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(57) Abstract

The present invention relates to proteinase K variants having a modified amino acid sequence of wild-type proteinase K amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type proteinase K (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the proteinase K variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type proteinase K. The present invention also relates to DNA sequences encoding such proteinase K variants. The present invention also relates to compositions comprising such proteinase K variants for cleaning a variety of surfaces.

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PROTEINASE K VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

TECHNICAL FIELD

The present invention relates to novel enzyme variants useful in a variety of cleaning compositions, and DNA sequences encoding such enzyme variants.

BACKGROUND

Enzymes make up the largest class of naturally occurring proteins. Each class of enzyme generally catalyzes (accelerates a reaction without being consumed) a different kind of chemical reaction. One class of enzymes known as proteases, are known for their ability to hydrolyze (break down a compound into two or more simpler compounds with the uptake of the H and OH parts of a water molecule on either side of the chemical bond cleaved) other proteins. This ability to hydrolyze proteins has been taken advantage of by incorporating naturally occurring and protein engineered proteases as an additive to laundry detergent preparations. Many stains on clothes are proteinaceous and wide-specificity proteases can substantially improve removal of such stains.

Unfortunately, the efficacy level of these proteins in their natural bacterial environment, frequently does not translate into the relatively unnatural wash environment. Specifically, protease characteristics such as thermal stability, pH stability, oxidative stability and substrate specificity are not necessarily optimized for utilization outside the natural environment of the enzyme.

The amino acid sequence of the protease determines the characteristics of the protease. A change of the amino acid sequence of the protease may alter the properties of the enzyme to varying degrees, or may even inactivate the enzyme, depending upon the location, nature and/or magnitude of the change in the amino acid sequence. Several approaches have been taken to alter the wild-type amino acid sequence of proteases in an attempt to improve their properties, with the goal of increasing the efficacy of the protease in the wash environment. These approaches include altering the amino acid sequence to enhance thermal stability and to

improve oxidation stability under quite diverse conditions.

Despite the variety of approaches described in the art, there is a continuing need for new effective variants of proteases useful for cleaning a variety of surfaces.

Objects of the Present Invention

It is an object of the present invention to provide Proteinase K enzyme variants having improved hydrolysis versus the wild-type of the enzyme.

It is also an object of the present invention to provide cleaning compositions comprising these subtilisin enzyme variants.

SUMMARY

The present invention relates to Proteinase K variants having a modified amino acid sequence of wild-type Proteinase K amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region and a fifth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Proteinase K (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Proteinase K variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Proteinase K. The present invention also relates to DNA sequences encoding such Proteinase K variants. The present invention also relates to compositions comprising such Proteinase K variants for cleaning a variety of surfaces.

DESCRIPTION

I. Proteinase K Variants

This invention pertains to subtilisin enzymes, in particular Proteinase K, that have been modified by mutating the various nucleotide sequences that code for the enzyme, thereby modifying the amino acid sequence of the enzyme. The modified subtilisin enzymes (hereinafter, "Proteinase K variants") of the present invention have decreased adsorption to and increased hydrolysis of an insoluble substrate as compared to the wild-type subtilisin. The present invention also pertains to DNA sequences encoding for such Proteinase K variants.

The subtilisin enzymes of this invention belong to a class of enzymes known as proteases. A protease is a catalyst for the cleavage of peptide bonds. One type of protease is a serine protease. A serine protease is

distinguished by the fact that there is an essential serine residue at the active site.

The observation that an enzyme's rate of hydrolysis of soluble substrates increases with enzyme concentration is well documented. It would therefore seem plausible that for surface bound substrates, such as is encountered in many cleaning applications, the rate of hydrolysis would increase with increasing surface concentration. This has been shown to be the case. (Brode, P.F. III and D. S. Rauch, LANGMUIR, "Subtilisin BPN': Activity on an Immobilized Substrate", Vol. 8, pp. 1325-1329 (1992)). In fact, a linear dependence of rate upon surface concentration was found for insoluble substrates when the surface concentration of the enzyme was varied. (Rubingh, D. N. and M. D. Bauer, "Catalysis of Hydrolysis by Proteases at the Protein-Solution Interface," in POLYMER SOLUTIONS, BLENDS AND INTERFACES, Ed. by I. Noda and D. N. Rubingh, Elsevier, p. 464 (1992)). Surprisingly, when seeking to apply this principle in the search for variant proteases which give better cleaning performance, we did not find that enzymes which adsorb more give better performance. In fact, we surprisingly determined the opposite to be the case: decreased adsorption by an enzyme to a substrate resulted in increased hydrolysis of the substrate (i.e., better cleaning performance).

While not wishing to be bound by theory, it is believed that improved performance, when comparing one variant to another, is a result of the fact that enzymes which adsorb less are also less tightly bound and therefore more highly mobile on the surface from which the insoluble protein substrate is to be removed. At comparable enzyme solution concentrations, this increased mobility is sufficient to outweigh any advantage that is conferred by delivering a higher concentration of enzyme to the surface.

The mutations described herein are designed to change (i.e., decrease) the adsorption of the enzyme to surface-bound soils. In Proteinase K, certain amino acids form exterior loops on the enzyme molecule. For purposes of discussion, these loops shall be referred to as first, second, third, fourth and fifth loop regions. Specifically, positions 64-71 form the first loop region; positions 95-107 form the second loop region; positions 133-140 form the third loop region; positions 160-170 form the fourth loop region; positions 190-194 form the fifth loop region; and positioins 203-223 form the sixth loop region (position numbering analagous to positions in the amino acid sequence for wild-type subtilisin Proteinase K

(SEQ ID NO:1)).

It is believed that these loop regions play a significant role in the adsorption of the enzyme molecule to a surface-bound peptide, and specific mutations in one or more of these loop regions will have a significant effect on this adsorption. While not wishing to be bound by theory, it is believed that the loop regions are important to the adsorption of the Proteinase K molecule for at least two reasons. First, the amino acids which comprise the loop regions can make close contacts with any surfaces to which the molecule is exposed. Second, the proximity of the loop regions to the active-site and binding pocket of the Proteinase K molecule gives them a role in the catalytically productive adsorption of the enzyme to surface-bound substrates (peptides/protein soils).

As used herein, "variant" means an enzyme having an amino acid sequence which differs from that of wild-type.

As used herein, "mutant Proteinase K DNA" means a DNA sequence coding for a Proteinase K variant.

As used herein, "wild-type Proteinase K" refers to an enzyme represented by SEQ ID NO:1. The amino acid sequence for Proteinase K is further described by Gunkel, F.A. and Gassen H.G., "Proteinase K from Tritirachium album Limber: Characterization of the Chromosomal Gene and Expression of the cDNA in Escherichia coli", EUR. J. BIOCHEM., Vol. 179, pp. 185-194 (1989), incorporated herein by reference.

As used herein, the term "Proteinase K wild-type amino acid sequence" encompasses SEQ ID NO:1 as well as SEQ ID NO:1 having modifications to the amino acid sequence other than at any of positions 64-71, 95-107, 133-140, 160-170, 190-194 and 203-223.

As used herein, "more hydrophilic amino acid" refers to any other amino acid having greater hydrophilicity than a subject amino acid with reference to the hydrophilicity table below. The following hydrophilicity table (Table 1) lists amino acids in descending order of increasing hydrophilicity (see Hopp, T.P., and Woods, K.R., "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, Vol. 78, pp. 3824-3828, 1981, incorporated herein by reference).

TABLE 1

Amino Acid	Hydrophilicity Value
Trp	-3.4
Phe	-2.5
Tyr	-2.3
Leu, Ile	-1.8
Val	-1.5
Met	-1.3
Cys	-1.0
Ala, His	-0.5
Thr	-0.4
Pro, Gly	-0.0
Gln, Asn	0.2
Ser	0.3
Arg^+, Lys^+, Glu^-, Asp^-	3.0

Table 1 also indicates which amino acids carry a charge (this characteristic being based on a pH of from about 8-9). The positively charged amino acids are Arg and Lys, the negatively charged amino acids are Glu and Asp, and the remaining amino acids are neutral. In a preferred embodiment of the present invention, the substituting amino acid is either neutral or negatively charged, more preferably negatively charged (i.e., Glu or Asp).

Therefore, for example, the statement "substitute Gln with an equally or more hydrophilic amino acid which is neutral or has a negative charge" means Gln would be substituted with Asn (which is equally hydrophilic to Gln), or Ser, Glu or Asp (which are more hydrophilic than Gln); each of which are neutral or have a negative charge, and have a greater hydrophilicity value as compared to Gln. Likewise, the statement "substitute Pro with a more hydrophilic amino acid which is neutral or has a negative charge" means Pro would be substituted with Gln, Asn, Ser, Glu or Asp.

In one embodiment of the present invention, the Proteinase K variant has a modified amino acid sequence of Proteinase K wild-type amino acid sequence, wherein the wild-type amino acid sequence comprises a substitution at one or more positions in one or more of the first loop region, the second loop region, the third loop region, the fourth loop region, the fifth loop region or the sixth loop region; whereby the Proteinase K variant has

decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Proteinase K.

In a preferred embodiment of the present invention, the substituting amino acid for one or more of the positions in one or more of the loop regions is, with reference to Table 1, neutral or negatively charged and equally or more hydrophylic, preferably more hydrophylic, than the amino acid at the subject position in the wild-type amino acid sequence.

A. Substitutions in the First Loop Region

When a substitution occurs in the first loop region, the substitution occurs at one or more of positions 64, 65, 66, 67, 68, 70 or 71.

When a substitution occurs at position 64, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 65, the substituting amino acid is Glu.

When a substitution occurs at position 66, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 67, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 68, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 70, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 71, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

B. Substitutions in the Second Loop Region

When a substitution occurs in the second loop region, the substitution occurs at one or more of positions 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106 or 107.

When a substitution occurs at position 95, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 96, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser , Thr or Val.

When a substitution occurs at position 97, the substituting amino acid is Glu.

When a substitution occurs at position 98, the substituting amino acid is Glu.

When a substitution occurs at position 99, the substituting amino acid

is Asp, Gln, Glu or Ser.

When a substitution occurs at position 100, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 101, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 102, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 103, the substituting amino acid is Asn, Asp, Glu or Ser.

When a substitution occurs at position 104, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 105, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 106, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 107, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

C. Substitutions in the Third Loop Region

When a substitution occurs in the third loop region, the substitution occurs at one or more of positions 133, 134, 135, 136, 137, 138, 139 or 140. wherein

When a substitution occurs at position 133, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 134, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 135, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 136, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 137, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 138, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 139, the substituting amino acid is Asp or Glu. and

When a substitution occurs at position 140, the substituting amino acid is Asp or Glu.

D. Substitutions in the Fourth Loop Region

When a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 160, 161, 162, 163, 164, 165, 166, 167, 168, 169 or 170. wherein

When a substitution occurs at position 160, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 161, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 162, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 163, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 164, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 165, the substituting amino acid is Glu.

When a substitution occurs at position 166, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 167, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 168, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 169, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val. and

When a substitution occurs at position 170, the substituting amino acid is Asp or Glu.

E. Substitutions in the Fifth Loop Region

When a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 190, 191, 192, 193 or 194. wherein

When a substitution occurs at position 190, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 191, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 192, the substituting amino

acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 193, the substituting amino acid is Asp or Glu. and

When a substitution occurs at position 194, the substituting amino acid is Asp, Gln, Glu or Ser. and

F. Substitutions in the Sixth Loop Region

When a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222 or 223. wherein

When a substitution occurs at position 203, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 206, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 207, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 209, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 210, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 211, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 212, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 213, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 215, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 216, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 217, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 218, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 219, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 220, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 221, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 222, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser. and

When a substitution occurs at position 223, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

G. Preparation of enzyme variants

Example 1

Mutant Proteinase K DNA

A phagemid ("PKP") containing the wild type Proteinase K gene is constructed. The 2.8 Kbp *Pvu* II restriction enzyme fragment of plasmid pUC119, (Vieira, J. and Messing, J.. "Production of Single-Stranded Plasmid DNA", 153 METHODS IN ENZYMOLOGY 3-11 (1989)) is cloned into the *Pvu* II site of plasmid pUB110 (Bacillus Genetic Stock Center, Columbus, OH 1E9). The pUC119-pUB110 hybrid plasmid is named pJMA601. Into pJMA601 is cloned the *Bacillus amyloliquefaciens* subtilisin gene. The subtilisin gene is modified to contain two *Bam*HI sites. One of the *Bam*HI sites is between DNA encoding Gly and Lys, the second and third amino acid residues of the pro region. The other *Bam*HI site follows the TAA stop sequence. A Proteinase K cDNA is amplified from RNA using reverse transcriptase and the polymerase chain reaction with oligonucleotides, each containing a BamHI site in addition to sequences identical to the Proteinase K cDNA. The amplified region consists of the DNA extending from the Ala at the beginning of the pro region of Proteinase K to the carboxy terminal Ala of the mature protease. The amplified segment is used to replace the DNA between two BamHI sites within the *Bacillus amyloliquefaciens* subtilisin gene. Phagemid PKP is transformed into *Escherichia coli* Ung$^-$

strain CJ236 and a single stranded uracil-containing DNA template is produced using the VCSM13 helper phage (Kunkel, T.A., J.D. Roberts and R.A. Zakour, "Rapid and efficient site-specific mutagenesis without phenotypic selection", METHODS IN ENZYMOLOGY, Vol. 154, pp. 367-382, (1987); as modified by Yuckenberg, P.D., F. Witney, J. Geisselsoder and J. McClary, "Site-directed in vitro mutagenesis using uracil-containing DNA and phagemid vectors", DIRECTED MUTAGENESIS - A PRACTICAL APPROACH, ed. M.J. McPherson, pp. 27-48, (1991); both of which are incorporated herein by reference). A single primer site-directed mutagenesis modification of the method of Zoller and Smith (Zoller, M.J., and M. Smith, "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA", NUCLEIC ACIDS RESEARCH, Vol. 10, pp. 6487-6500, (1982), incorporated herein by reference) is used to produce all mutants (basically as presented by Yuckenberg, et al., 1991, above). Oligonucleotides are made using an Applied Biosystem Inc. 380B DNA synthesizer. Mutagenesis reaction products are transformed into *Escherichia coli* strain MM294 (American Type Culture Collection *E. coli.* 33625). All mutants are confirmed by DNA sequencing and the isolated DNA is transformed into the *Bacillus subtilis* expression strain BG2036 (Yang, M. Y., E. Ferrari and D. J. Henner, (1984), "Cloning of the Neutral Protease Gene of *Bacillus subtilis* and the Use of the Cloned Gene to Create an In Vitro-derived Deletion Mutation", JOURNAL OF BACTERIOLOGY, Vol. 160, pp. 15-21). For some of the loop mutants a modified PKP with a frameshift-stop codon mutation in the corresponding loop is used to produce the uracil template. Oligonucleotides are designed to restore the proper reading frame and to encode for random substitutions at positions 64, 65, 66, 67, 68, 70, 71, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 133, 134, 135, 136, 137, 138, 139, 140, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 190, 191, 192, 193, 194, 203, 204, 205, 206, 207 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222 or 223 (equimolar and/or variable mixtures of all four nucleotides for all three bases at these codons). Mutations that correct for the frameshift-stop and produce a functional enzyme are identified by their ability to digest casein. The random substitutions are determined by DNA sequencing.

Example 2

Fermentation

The *Bacillus subtilis* cells (BG2036) containing a subtilisin mutant of interest are grown to mid-log phase in a one liter culture of LB-glucose broth and inoculated into a Biostat ED fermenter (B. Braun Biotech, Inc., Allentown, Pennsylvania) in a total volume of 10 liters. The fermentation media contains Yeast Extract, starch, antifoam, buffers and trace minerals (see FERMENTATION: A PRACTICAL APPROACH, Ed. B. McNeil and L. M. Harvey, 1990). The broth is kept at a constant pH of 7.0 during the fermentation run. Chloramphenical is added for antibiotic selection of mutagenized plasmid. The cells are grown overnight at 37°C to an A_{600} of about 60 and harvested.

Example 3

Purification

The fermentation broth is taken through the following steps to obtain pure enzyme. The broth is cleared of *Bacillus subtilis* cells by centrifugation, and clarified by removing fine particulates with a 100K cutoff membrane. This is followed by concentration on a 10K cutoff membrane, and flow dialysis to reduce the ionic strength and adjust the pH to 5.5 using 0.025M MES buffer (2-(*N*-morpholino)ethanesulfonic acid). The enzyme is further purified by loading it onto either a cation exchange chromatography column or an affinity adsorption chromatography column and eluting it from the column with a NaCl or a propylene glycol gradient (*see* Scopes, R. K., PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, Springer-Verlag, New York (1984), incorporated herein by reference).

The *p*NA assay (DelMar, E.G., C. Largman, J.W. Brodrick and M.C. Geokas, ANAL. BIOCHEM., Vol. 99, pp. 316-320, (1979), incorporated herein by reference) is used to determine the active enzyme concentration for fractions collected during gradient elution. This assay measures the rate at which *p*-nitroaniline is released as the enzyme hydrolyzes the soluble synthetic substrate, succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (sAAPF-*p*NA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nm are used to determine the total protein concentration. The active enzyme/total-protein ratio gives the enzyme purity, and is used to identify fractions to be pooled for the stock solution.

To avoid autolysis of the enzyme during storage, an equal weight of propylene glycol is added to the pooled fractions obtained from the chromatography column. Upon completion of the purification procedure the purity of the stock enzyme solution is checked with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and the absolute enzyme concentration is determined via an active site titration method using trypsin inhibitor type II-T: turkey egg white purchased from Sigma Chemical Company (St. Louis, Missouri). The measured conversion factors will show which changes made in the enzyme molecule at the various positions result in the enzyme variant having increased activity over the wild-type, against the soluble substrate *p*NA.

In preparation for use, the enzyme stock solution is eluted through a Sephadex-G25 (Pharmacia, Piscataway, New Jersey) size exclusion column to remove the propylene glycol and exchange the buffer. The MES buffer in the enzyme stock solution is exchanged for 0.1 M Tris buffer (Tris(hydroxymethyl-aminomethane) containing 0.01M $CaCl_2$ and pH adjusted to 8.6 with HCl. All experiments are carried out at pH 8.6 in Tris buffer thermostated at 25°C.

H. Characterization of enzyme variants

Example 4

Model Surface Preparation

Aminopropyl controlled pore glass (CPG) purchased from CPG Inc. (Fairfield, New Jersey) is used as a support for covalently attaching the sAAPF-*p*NA substrate purchased from Bachem, Inc. (Torrence, California). The reaction is carried out in dimethyl sulfoxide and (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride) (EDC) is used as a coupling agent. Upon completion (monitored by *p*NA assay), the excess solvent is removed, and the CPG:sAAPF-*p*NA is rinsed with dimethyl sulfoxide (DMSO) and doubly-distilled water. This is followed by oven drying with a N_2 purge at about 70°C. The reaction scheme and preparation of the immobilized substrate are conducted as described by Brode, P.F. III, and D.S. Rauch, "Subtilisin BPN': Activity on an Immobilized Substrate," LANGMUIR, Vol. 8, p. 1325-1329, (1992), incorporated herein by reference.

The CPG surface will have 62,000 ± 7,000 *p*NA molecules/μm^2. The surface area will remain unchanged from the value of 50.0m^2/g reported by CPG Inc. for the CPG as received. This suggests that the procedure used

to add sAAPF-*p*NA to CPG does not damage the porous structure (mean diameter is 486 Å).

Example 5

Surface Hydrolysis Assay

Using CPG:sAAPF-*p*NA, adsorption of an enzyme variant and hydrolysis of a CPG-bound peptide can be measured in a single experiment. A small volume of enzyme variant stock solution is added to a flask containing Tris buffer and CPG:sAAPF-*p*NA which has been degassed. The flask is shaken on a wrist-action shaker for a period of 90 minutes during which the shaker is stopped at various time intervals (for example, every 2 minutes during the early stages of adsorption hydrolysis - e.g., the first 20 minutes - and every 10 minutes towards the end of the experiment). The CPG:sAAPF-*p*NA is allowed to settle and the solution is sampled. Both the experimental procedure and the calculation of the adsorption and hydrolysis are conducted as described by Brode *et al.*, 1992, above.

All enzymes are monitored for stability against autolysis and should show no appreciable autolytic loss over the time course of this experiment. Therefore, enzyme adsorption can be determined by measuring solution depletion. The difference between the initial enzyme variant concentration and the concentration measured at each individual time point gives the amount of enzyme variant adsorbed. The amount of *p*NA hydrolyzed from the surface is measured by taking an absorbance reading on an aliquot of the sample at 410 nm. The total amount of *p*NA hydrolyzed is calculated by adding the amount sampled and the amount remaining in the flask. This value is corrected by subtracting the amount of *p*NA that is hydrolyzed by Tris buffer at pH 8.6 when no enzyme is present. This base-hydrolysis ranges from 7-29% of the total hydrolysis depending on the efficiency of the enzyme.

Example 6

Soluble Substrate Kinetic Analysis

The rates of hydrolysis of the soluble substrate sAAPF-*p*NA are monitored by measuring the adsorbance increase as a function of time at 410 nm on a DU-70 spectrophotometer. The enzyme concentration is held constant and is prepared to be in the range of 6-10 nanomolar while the substrate concentration is varied from 90-700 μM sAAPF-*p*NA for each kinetic determination. An adsorbance data point is taken each second over

a period of 900 seconds and the data are transferred to a LOTUS™ spreadsheet (Lotus Development Corporation, Cambridge, Massachusetts). Analysis for kinetic parameters is conducted by the standard Lineweaver Burk analysis in which the data in the initial part of the run (generally the first minute) are fit to a linear regression curve to give v_o. The v_o and s_o data are plotted in the standard inverse fashion to give K_M and k_{cat}.

I. Example Proteinase K variants

Proteinase K variants of the present invention which have decreased adsorption to and increased hydrolysis of surface bound substrates are exemplified in Tables 2-36, below. In describing the specific mutations, the original amino acid occurring in wild-type is given first, the position number second, and the substituted amino acid third.

TABLE 2

Loop 1 - Single Mutation Variants
Arg64Asp
Arg64Glu
Asp65Glu
Gly66Asn
Gly66Asp
Gly66Gln
Gly66Glu
Gly66Pro
Gly66Ser
Asn67Asp
Asn67Gln
Asn67Glu
Asn67Ser
Gly68Asn
Gly68Asp
Gly68Gln
Gly68Glu
Gly68Pro
Gly68Ser
Gly70Asn
Gly70Asp
Gly70Gln
Gly70Glu
Gly70Pro
Gly70Ser
Thr71Asn
Thr71Asp
Thr71Gln
Thr71Glu
Thr71Gly
Thr71Pro
Thr71Ser

TABLE 3

Loop 1 - Double Mutation Variants
Asn67Ser + Thr71Asp
Gly66Ser + Thr71Ser
Arg64Asp + Thr71Asn
Arg64Asp + Gly68Ser
Gly66Glu + Gly70Ser
Arg64Glu + Gly68Ser
Arg64Glu + Gly66Gln
Asn67Glu + Gly70Asn
Asn67Gln + Thr71Asp
Gly66Ser + Gly70Asn
Asp65Glu + Gly70Ser
Arg64Glu + Gly70Asn
Gly66Pro + Thr71Asn
Gly70Gln + Thr71Gln
Gly68Glu + Gly70Asn
Asp65Glu + Thr71Ser
Gly68Asp + Gly70Gln
Asp65Glu + Gly68Gln
Gly70Gln + Thr71Asp
Gly68Gln + Gly70Pro
Asn67Asp + Gly70Gln
Gly66Ser + Asn67Gln
Asp65Glu + Gly68Asn
Gly68Asp + Gly70Pro
Gly66Gln + Thr71Pro
Asp65Glu + Thr71Pro
Arg64Asp + Gly68Asn
Gly68Asn + Thr71Glu
Gly66Asn + Thr71Glu
Gly68Ser + Thr71Asp
Gly68Ser + Thr71Gly
Gly66Ser + Gly68Gln
Gly66Gln + Gly70Ser
Gly66Glu + Gly70Pro
Arg64Glu + Gly70Pro
Asp65Glu + Thr71Gly
Arg64Glu + Thr71Gly
Asp65Glu + Gly70Pro
Arg64Asp + Gly68Gln
Arg64Glu + Gly68Pro
Gly66Asn + Gly70Ser

TABLE 4

Loop 1 - Triple Mutation Variants
Asn67Ser + Gly68Asp + Gly70Ser
Asn67Asp + Gly70Asn + Thr71Gly
Arg64Glu + Gly66Asn + Gly70Pro

Gly66Asp + Gly68Ser + Gly70Ser
Asp65Glu + Gly70Gln + Thr71Gln
Gly66Glu + Gly68Pro + Gly70Asn
Arg64Asp + Gly66Ser + Gly70Gln
Gly66Gln + Asn67Gln + Gly68Glu
Gly66Glu + Asn67Ser + Thr71Pro
Gly66Asn + Gly68Pro + Thr71Gln
Arg64Glu + Gly66Gln + Gly68Gln
Gly66Asn + Gly68Ser + Gly70Pro
Arg64Asp + Gly70Gln + Thr71Gly
Asp65Glu + Gly68Gln + Gly70Ser
Arg64Glu + Gly66Pro + Gly68Ser
Gly66Gln + Asn67Gln + Thr71Gly
Arg64Glu + Gly68Pro + Thr71Ser
Asn67Asp + Gly70Ser + Thr71Gly
Arg64Glu + Gly70Pro + Thr71Gln
Asp65Glu + Gly68Gln + Gly70Gln
Gly66Pro + Gly68Gln + Thr71Pro
Gly66Glu + Asn67Gln + Gly70Pro
Gly66Gln + Gly70Ser + Thr71Gln
Asp65Glu + Gly68Gln + Gly70Asn
Arg64Glu + Gly66Ser + Thr71Pro
Asp65Glu + Gly68Ser + Gly70Asn
Asp65Glu + Asn67Ser + Gly70Ser
Arg64Glu + Gly66Ser + Gly70Pro
Asp65Glu + Gly70Gln + Thr71Gly
Asp65Glu + Gly68Asn + Gly70Asn
Gly68Asn + Gly70Pro + Thr71Asp
Arg64Asp + Gly68Ser + Gly70Pro
Gly66Asn + Gly68Glu + Gly70Gln
Asn67Glu + Gly68Gln + Gly70Asn
Gly66Glu + Gly68Pro + Gly70Gln
Asn67Asp + Gly68Glu + Gly70Ser
Asn67Asp + Gly68Asp + Gly70Asn
Arg64Asp + Asp65Glu + Asn67Ser
Arg64Asp + Asp65Glu + Gly70Pro
Arg64Glu + Asp65Glu + Asn67Gln

TABLE 5

Loop 1 - Quadruple Mutation Variants

Asp65Glu + Gly68Pro + Gly70Asn + Thr71Asn
Asp65Glu + Asn67Gln + Gly68Gln + Gly70Ser
Gly66Glu + Asn67Gln + Gly68Asn + Gly70Gln
Asp65Glu + Gly66Asn + Gly70Ser + Thr71Gln
Asn67Gln + Gly68Glu + Gly70Gln + Thr71Gly
Arg64Glu + Gly66Gln + Gly70Asn + Thr71Gln
Gly66Pro + Gly68Ser + Gly70Asn + Thr71Gln
Arg64Glu + Gly68Pro + Gly70Gln + Thr71Pro
Gly66Glu + Gly68Pro + Gly70Asn + Thr71Pro
Asp65Glu + Gly66Glu + Asn67Ser + Gly70Pro
Asp65Glu + Gly66Asp + Gly68Asn + Thr71Ser

Asp65Glu + Gly66Glu + Gly68Pro + Gly70Ser
Asp65Glu + Gly66Glu + Gly70Asn + Thr71Gln
Arg64Asp + Asp65Glu + Asn67Gln + Gly70Asn
Arg64Asp + Asp65Glu + Gly70Pro + Thr71Gln
Gly66Glu + Asn67Asp + Gly68Ser + Gly70Ser
Gly66Glu + Asn67Glu + Gly70Asn + Thr71Ser
Asp65Glu + Gly66Asp + Asn67Glu + Gly68Ser
Arg64Glu + Asp65Glu + Gly66Asp + Gly68Pro
Gly66Glu + Asn67Asp + Gly68Asp + Gly70Asn
Asp65Glu + Asn67Glu + Gly68Ser + Gly70Gln
Asp65Glu + Asn67Asp + Gly68Gln + Gly70Ser
Asp65Glu + Asn67Glu + Gly68Gln + Gly70Asn
Asp65Glu + Gly66Asn + Asn67Glu + Gly70Pro
Asp65Glu + Gly66Asp + Gly68Asp + Thr71Asn
Asp65Glu + Gly66Glu + Asn67Gln + Gly68Asp

TABLE 6

Loop 2 - Single Mutation Variants
Val 95Ala
Val 95Asn
Val 95Asp
Val 95Cys
Val 95Gln
Val 95Glu
Val 95Gly
Val 95His
Val 95Met
Val 95Pro
Val 95Ser
Val 95Thr
Leu 96Ala
Leu 96Asn
Leu 96Asp
Leu 96Cys
Leu 96Gln
Leu 96Glu
Leu 96Gly
Leu 96His
Leu 96Ile
Leu 96Met
Leu 96Pro
Leu 96Ser
Leu 96Thr
Leu 96Val
Asp 97Glu
Asp 98Glu
Asn 99Asp
Asn 99Gln
Asn 99Glu
Asn 99Ser
Gly100Asn

Gly100Asp
Gly100Gln
Gly100Glu
Gly100Pro
Gly100Ser
Ser101Asp
Ser101Glu
Gly102Asn
Gly102Asp
Gly102Gln
Gly102Glu
Gly102Pro
Gly102Ser
Gln103Asn
Gln103Asp
Gln103Glu
Gln103Ser
Tyr104Ala
Tyr104Asn
Tyr104Asp
Tyr104Cys
Tyr104Gln
Tyr104Glu
Tyr104Gly
Tyr104His
Tyr104Ile
Tyr104Leu
Tyr104Met
Tyr104Pro
Tyr104Ser
Tyr104Thr
Tyr104Val
Ser105Asp
Ser105Glu
Thr106Asn
Thr106Asp
Thr106Gln
Thr106Glu
Thr106Gly
Thr106Pro
Thr106Ser
Ile107Ala
Ile107Asn
Ile107Asp
Ile107Cys
Ile107Gln
Ile107Glu
Ile107Gly
Ile107His
Ile107Leu
Ile107Met
Ile107Pro
Ile107Ser

Ile107Thr
Ile107Val

TABLE 7

Loop 2 - Double Mutation Variants

Val 95Gln + Ser101Glu
Tyr104His + Ile107Asp
Val 95Glu + Ile107Asn
Asn 99Gln + Gln103Asp
Leu 96Gly + Ser105Glu
Val 95Met + Gln103Asp
Ser101Asp + Ile107Thr
Val 95His + Asp 97Glu
Asp 98Glu + Tyr104Leu
Leu 96Cys + Asp 97Glu
Gln103Glu + Tyr104Gln
Gly102Ser + Tyr104Gly
Leu 96Ala + Thr106Pro
Thr106Gln + Ile107Asn
Asn 99Gln + Ile107Asp
Asn 99Glu + Thr106Gly
Gly102Pro + Gln103Asp
Asn 99Asp + Tyr104Thr
Leu 96Ile + Ser101Glu
Val 95Gly + Gln103Ser
Tyr104Leu + Ser105Glu
Gly102Asn + Tyr104Pro
Leu 96Asp + Ile107Leu
Asp 98Glu + Gly102Asn
Leu 96Pro + Gly100Glu
Ser101Asp + Thr106Ser
Gly100Ser + Gln103Asn
Gly102Pro + Tyr104Thr
Leu 96Glu + Ile107Leu
Leu 96Ile + Gly100Asp
Gly100Pro + Ile107Ser
Asp 97Glu + Asn 99Gln
Asp 97Glu + Ile107Cys
Gly102Ser + Gln103Glu
Gly100Asn + Tyr104Asp
Gly100Pro + Thr106Asp
Val 95Pro + Asp 97Glu
Val 95Thr + Gly100Glu
Thr106Pro + Ile107Glu
Ser101Asp + Gly102Asn
Ser105Glu + Ile107Thr
Asn 99Gln + Ser105Asp
Gln103Ser + Ile107Met
Leu 96Asp + Thr106Gly
Val 95Thr + Gly102Asp
Val 95Ala + Ser105Asp

Gly100Asn + Gln103Ser	
Gly102Asp + Thr106Gln	
Leu 96Asn + Ser105Asp	
Gly100Glu + Gly102Pro	
Gln103Asp + Ile107His	
Tyr104Ala + Ile107Pro	
Asp 97Glu + Ile107Pro	
Asn 99Glu + Tyr104Asn	
Val 95Thr + Asp 98Glu	
Gly100Gln + Thr106Gly	
Asn 99Glu + Gln103Ser	
Val 95His + Ser105Asp	
Gly102Pro + Ile107Ala	
Asp 97Glu + Gly100Gln	

TABLE 8

Loop 2 - Triple Mutation Variants

Val 95Gln +	Leu 96Thr +	Ser101Glu
Ser101Asp +	Thr106Ser +	Ile107Leu
Val 95Asn +	Leu 96Asn +	Asp 98Glu
Gln103Glu +	Tyr104Ser +	Ile107Ser
Val 95Cys +	Leu 96Glu +	Gly102Ser
Val 95His +	Tyr104Thr +	Ser105Glu
Leu 96Pro +	Asn 99Asp +	Gln103Ser
Val 95Ser +	Leu 96Asp +	Gln103Ser
Leu 96Cys +	Gly102Gln +	Ile107Leu
Val 95Ser +	Leu 96His +	Thr106Pro
Leu 96Gln +	Gly102Pro +	Ser105Asp
Leu 96Met +	Asn 99Ser +	Ser105Asp
Leu 96His +	Ser101Asp +	Tyr104Val
Asn 99Asp +	Gly100Asn +	Gly102Ser
Val 95Gln +	Gly100Asp +	Thr106Pro
Leu 96Asn +	Tyr104Glu +	Ile107Gln
Asn 99Asp +	Gly102Ser +	Tyr104His
Val 95Met +	Leu 96Gly +	Gly100Pro
Val 95Ala +	Asp 98Glu +	Asn 99Ser
Asp 97Glu +	Tyr104Thr +	Ile107His
Leu 96Thr +	Gly102Gln +	Gln103Asp
Tyr104Met +	Thr106Glu +	Ile107Leu
Gly100Ser +	Gln103Asn +	Tyr104Ile
Gln103Asn +	Tyr104Pro +	Thr106Glu
Val 95Met +	Asp 98Glu +	Asn 99Gln
Leu 96His +	Asp 97Glu +	Ile107Met
Gly100Asn +	Gln103Ser +	Ser105Asp
Gly102Asn +	Gln103Glu +	Thr106Pro
Val 95Met +	Gly100Ser +	Gly102Asp
Val 95Asp +	Leu 96Met +	Tyr104Asn
Gln103Glu +	Tyr104Cys +	Ile107Asn
Val 95Met +	Leu 96Ile +	Tyr104Met
Val 95Ser +	Ser101Glu +	Thr106Gly
Val 95Pro +	Asn 99Asp +	Gly100Ser

Gly100Ser + Gln103Glu + Ile107Val
Gln103Asp + Tyr104Val + Ile107His
Gly100Gln + Tyr104Met + Thr106Asp
Asn 99Asp + Gly100Ser + Tyr104His
Ser101Asp + Gly102Asp + Gln103Ser
Asp 98Glu + Asn 99Asp + Tyr104Ala
Asp 98Glu + Asn 99Glu + Gly100Gln
Gly102Asp + Gln103Asp + Thr106Gln
Tyr104Glu + Ser105Asp + Thr106Pro
Asp 97Glu + Asp 98Glu + Tyr104Thr
Asp 97Glu + Asp 98Glu + Asn 99Asp
Asn 99Asp + Gly100Asp + Ser101Glu
Gln103Glu + Tyr104Asp + Ile107Glu
Leu 96Glu + Asp 97Glu + Gly102Asp
Leu 96Glu + Ser101Asp + Gln103Asn
Asp 97Glu + Gly100Asp + Ile107His
Asp 97Glu + Asn 99Ser + Gly100Asp
Gln103Asp + Ser105Glu + Thr106Asn
Leu 96Ser + Ser105Asp + Ile107Asp
Asp 97Glu + Ser101Glu + Gln103Ser
Asp 97Glu + Ser101Glu + Tyr104Met
Asn 99Asp + Gly100Ser + Ser101Asp
Gln103Asp + Tyr104His + Ile107Glu
Asp 97Glu + Gly102Glu + Tyr104Pro
Val 95Asp + Gly102Glu + Tyr104Gly
Val 95Asp + Asp 97Glu + Ile107Asn

TABLE 9

Loop 2 - Quadruple Mutation Variants

Val 95Gln + Asp 97Glu + Gly100Ser + Ile107Gln
Val 95Ser + Leu 96Pro + Asp 97Glu + Gln103Asn
Leu 96Gln + Gly100Ser + Ser101Asp + Thr106Asn
Val 95Cys + Asp 97Glu + Gly100Gln + Gly102Ser
Leu 96Pro + Gly102Asn + Ser105Asp + Ile107Gln
Val 95Ser + Gly100Gln + Ser101Glu + Ile107Asn
Leu 96Met + Ser101Glu + Gln103Asn + Thr106Pro
Asn 99Asp + Gly100Pro + Gly102Ser + Tyr104Ala
Gly100Pro + Gly102Pro + Thr106Gly + Ile107Glu
Val 95Ser + Gly100Gln + Ser101Asp + Gly102Pro
Val 95Gly + Leu 96Val + Thr106Pro + Ile107Asp
Val 95Gln + Leu 96Val + Asn 99Asp + Ile107Thr
Leu 96Ser + Asp 97Glu + Thr106Gln + Ile107Met
Leu 96Thr + Asn 99Gln + Tyr104Val + Ile107Cys
Asn 99Glu + Gly100Gln + Gly102Asn + Gln103Ser
Val 95Gly + Ser101Glu + Gln103Ser + Thr106Gly
Val 95Asn + Leu 96Gln + Asp 98Glu + Gly100Asn
Val 95Cys + Gly102Pro + Tyr104Val + Ile107Asp
Val 95Met + Leu 96Met + Gly102Glu + Tyr104Thr
Val 95Asp + Leu 96Cys + Thr106Ser + Ile107Gly
Leu 96Met + Gly100Glu + Ser101Glu + Tyr104Cys
Asp 98Glu + Asn 99Glu + Gly102Asn + Ile107Ser

Val 95Ser + Asp 97Glu + Asp 98Glu + Asn 99Ser
Val 95Gly + Asp 97Glu + Asp 98Glu + Tyr104Ile
Leu 96Gly + Asp 97Glu + Asp 98Glu + Asn 99Asp
Asp 98Glu + Asn 99Glu + Gly100Glu + Thr106Gly
Leu 96Glu + Asp 97Glu + Ser101Asp + Tyr104Ile
Asp 97Glu + Gly100Asp + Ser101Glu + Tyr104Leu
Asp 97Glu + Asp 98Glu + Gly100Glu + Tyr104Met
Gly100Glu + Ser101Asp + Gly102Asp + Ile107Gly
Val 95Met + Leu 96Glu + Asp 97Glu + Gly102Asp
Asp 97Glu + Gly100Glu + Tyr104Ala + Thr106Gln
Asp 97Glu + Asn 99Glu + Tyr104His + Thr106Gly
Gly102Ser + Gln103Asp + Ser105Asp + Thr106Ser
Asp 97Glu + Ser101Asp + Gly102Pro + Tyr104Ile
Asn 99Asp + Gly100Gln + Ser101Asp + Thr106Pro
Asn 99Asp + Gly100Ser + Ser101Asp + Ile107Cys
Gly102Ser + Gln103Glu + Ser105Asp + Ile107Asp
Asp 98Glu + Gly100Glu + Ser101Glu + Gly102Gln
Val 95Gly + Asp 98Glu + Asn 99Glu + Ser101Asp
Asp 97Glu + Gly102Asp + Gln103Ser + Ile107Met
Leu 96Ser + Asp 97Glu + Gly100Glu + Gly102Glu
Val 95Thr + Leu 96Glu + Asp 98Glu + Ser101Asp
Gly102Glu + Tyr104Ala + Ser105Asp + Thr106Asp
Leu 96Met + Ser101Asp + Gln103Glu + Ile107Gln
Ser101Asp + Gln103Asp + Tyr104Ser + Ile107Thr
Asp 98Glu + Gly100Asn + Ser101Glu + Tyr104Val
Val 95Glu + Gly102Glu + Gln103Asn + Tyr104Glu
Val 95Thr + Ser101Glu + Gln103Asp + Ile107Glu
Ser101Glu + Gln103Asp + Tyr104Met + Ile107Glu
Val 95Glu + Ser101Asp + Tyr104His + Ile107Glu
Val 95Gly + Asp 97Glu + Gly102Glu + Thr106Glu
Val 95Ser + Leu 96Asp + Asn 99Gln + Gln103Glu
Leu 96Glu + Gly102Asn + Tyr104Gln + Thr106Glu
Val 95Asp + Asp 98Glu + Gly100Asp + Thr106Gly
Leu 96Glu + Gly102Asp + Gln103Ser + Ser105Glu
Gly100Ser + Ser101Glu + Gln103Asp + Ser105Asp
Val 95Met + Gly100Glu + Gly102Asp + Ile107Asp
Leu 96Ala + Asp 97Glu + Ser101Asp + Ile107Asp
Leu 96Gln + Asp 97Glu + Gln103Asp + Ile107Glu

TABLE 10

Loop 3 - Single Mutation Variants
Leu133Ala
Leu133Asn
Leu133Asp
Leu133Cys
Leu133Gln
Leu133Glu
Leu133Gly
Leu133His
Leu133Ile
Leu133Met

Leu133Pro
Leu133Ser
Leu133Thr
Leu133Val
Gly134Asn
Gly134Asp
Gly134Gln
Gly134Glu
Gly134Pro
Gly134Ser
Gly135Asn
Gly135Asp
Gly135Gln
Gly135Glu
Gly135Pro
Gly135Ser
Gly136Asn
Gly136Asp
Gly136Gln
Gly136Glu
Gly136Pro
Gly136Ser
Tyr137Ala
Tyr137Asn
Tyr137Asp
Tyr137Cys
Tyr137Gln
Tyr137Glu
Tyr137Gly
Tyr137His
Tyr137Ile
Tyr137Leu
Tyr137Met
Tyr137Pro
Tyr137Ser
Tyr137Thr
Tyr137Val
Ser138Asp
Ser138Glu
Ser139Asp
Ser139Glu
Ser140Asp
Ser140Glu

TABLE 11

Loop 3 - Double Mutation Variants

Gly134Asn + Ser140Asp
Leu133Ala + Gly135Glu
Leu133Thr + Ser139Asp
Leu133Gln + Ser140Asp
Gly136Gln + Ser138Glu

Gly134Ser + Ser138Asp
Gly136Pro + Ser139Asp
Gly135Asn + Ser138Asp
Gly135Glu + Gly136Pro
Leu133Ile + Ser140Asp
Leu133Cys + Ser140Asp
Gly134Asn + Gly135Pro
Leu133Cys + Gly136Asp
Tyr137Asn + Ser139Asp
Gly136Asn + Ser140Glu
Gly134Glu + Gly135Pro
Tyr137Met + Ser140Asp
Gly135Gln + Ser139Glu
Tyr137Thr + Ser138Glu
Leu133Asn + Ser139Glu
Gly135Ser + Gly136Gln
Leu133Gly + Gly136Ser
Leu133Ala + Tyr137His
Leu133Val + Gly136Glu
Tyr137Ile + Ser139Asp
Leu133Ile + Tyr137Gln
Gly136Ser + Ser140Asp
Gly134Asn + Ser138Glu
Gly134Ser + Ser138Glu
Gly135Ser + Ser138Asp
Gly136Ser + Ser140Glu
Gly136Ser + Ser138Glu
Leu133Glu + Gly134Asn
Leu133Glu + Gly135Gln
Gly135Asn + Tyr137Glu
Tyr137Thr + Ser139Glu
Tyr137Ala + Ser139Glu
Gly134Gln + Tyr137Gly
Gly135Pro + Tyr137His
Leu133Pro + Ser138Glu
Leu133Thr + Gly135Ser
Gly136Pro + Ser138Glu
Gly134Ser + Ser140Asp
Leu133Met + Tyr137Ala
Tyr137Val + Ser138Glu
Gly134Pro + Ser140Glu
Leu133Thr + Tyr137Ser
Gly135Glu + Gly136Asn
Leu133Ala + Ser140Glu
Gly134Gln + Ser139Glu
Leu133Pro + Tyr137Gly
Leu133Val + Gly136Ser
Gly136Asp + Tyr137Pro
Gly136Asn + Tyr137Val
Leu133Ala + Ser138Asp
Gly135Gln + Gly136Pro
Tyr137Met + Ser138Glu
Leu133Gln + Tyr137Asp

Tyr137Ser + Ser138Glu
Gly134Asp + Gly135Pro

TABLE 12

Loop 3 - Triple Mutation Variants

Leu133Cys + Gly135Gln + Tyr137Glu
Leu133Ile + Gly134Ser + Gly136Glu
Leu133Gly + Gly135Glu + Tyr137Val
Leu133Gln + Gly135Gln + Ser139Asp
Leu133Asp + Gly135Pro + Tyr137Asn
Leu133Val + Gly135Gln + Gly136Ser
Gly135Asn + Tyr137Ile + Ser139Glu
Leu133Met + Gly136Pro + Ser140Asp
Gly134Pro + Gly136Pro + Ser138Glu
Gly135Asn + Tyr137Met + Ser138Asp
Leu133Ser + Gly136Asn + Tyr137Val
Gly134Glu + Gly135Ser + Gly136Pro
Gly136Asn + Tyr137Met + Ser140Glu
Leu133Ile + Gly136Pro + Tyr137Glu
Leu133Met + Gly136Gln + Tyr137Gln
Leu133Val + Gly135Pro + Ser139Asp
Leu133Asp + Gly135Gln + Tyr137Cys
Gly135Pro + Tyr137Met + Ser138Glu
Gly136Gln + Tyr137Gly + Ser140Asp
Leu133Ser + Gly134Pro + Ser140Asp
Leu133Thr + Gly134Asp + Gly135Pro
Leu133Ile + Gly135Asn + Ser139Glu
Gly135Glu + Gly136Pro + Tyr137His
Gly135Asn + Gly136Pro + Ser140Glu
Leu133Met + Gly134Ser + Gly136Gln
Gly134Pro + Gly135Asn + Ser140Glu
Gly135Gln + Gly136Gln + Ser138Asp
Gly136Ser + Tyr137Met + Ser138Glu
Gly134Pro + Gly136Ser + Ser139Asp
Gly134Ser + Tyr137Thr + Ser138Asp
Leu133Thr + Gly134Pro + Ser140Asp
Gly134Gln + Gly135Pro + Ser140Glu
Gly134Pro + Tyr137His + Ser138Asp
Leu133Met + Gly134Gln + Ser139Asp
Leu133Ser + Gly134Asn + Ser138Glu
Gly136Gln + Tyr137Ala + Ser139Glu
Leu133Ile + Gly136Asn + Ser139Asp
Leu133Ala + Gly135Asp + Gly136Pro
Leu133Asn + Ser138Asp + Ser139Glu
Gly136Asn + Ser138Asp + Ser139Asp
Gly135Asn + Ser138Asp + Ser139Glu
Leu133Asp + Gly134Asp + Tyr137His
Leu133Asp + Gly134Glu + Gly135Pro
Leu133Gly + Gly136Glu + Tyr137Asp
Tyr137Ser + Ser139Glu + Ser140Asp
Gly134Gln + Ser139Asp + Ser140Asp

Gly136Asn + Ser139Asp + Ser140Glu
Tyr137Pro + Ser139Asp + Ser140Asp
Gly135Ser + Tyr137Glu + Ser138Glu
Ser138Asp + Ser139Asp + Ser140Asp
Tyr137Glu + Ser138Asp + Ser139Glu
Tyr137Ala + Ser138Asp + Ser140Asp
Tyr137Thr + Ser138Asp + Ser140Glu
Tyr137Gln + Ser138Asp + Ser140Asp
Leu133Thr + Ser138Asp + Ser140Glu
Gly135Ser + Ser138Asp + Ser140Glu
Gly136Gln + Ser138Asp + Ser140Asp
Gly135Ser + Ser138Glu + Ser140Asp
Gly136Gln + Ser138Glu + Ser140Asp
Leu133Val + Ser138Glu + Ser140Glu

TABLE 13

Loop 3 - Quadruple Mutation Variants

Leu133Ala + Gly134Asn + Tyr137Ser + Ser138Asp
Gly134Glu + Gly135Asn + Gly136Asn + Tyr137Cys
Leu133Cys + Gly135Asn + Gly136Gln + Ser138Asp
Leu133Pro + Gly134Asn + Gly135Ser + Ser139Asp
Leu133Val + Gly134Gln + Gly136Gln + Ser140Glu
Leu133Asp + Gly134Gln + Gly136Gln + Tyr137Thr
Leu133His + Gly135Gln + Gly136Pro + Tyr137Ser
Leu133Gly + Gly134Ser + Tyr137Ala + Ser138Asp
Leu133Gln + Gly134Gln + Gly135Asn + Ser140Glu
Leu133Ser + Gly135Ser + Tyr137Pro + Ser139Glu
Leu133Asn + Gly134Asn + Gly136Glu + Tyr137Cys
Leu133Met + Gly134Asn + Gly135Gln + Ser138Glu
Leu133Asn + Gly134Ser + Tyr137Val + Ser138Glu
Gly135Asn + Gly136Pro + Tyr137Cys + Ser139Asp
Leu133Ile + Gly135Asp + Gly136Asn + Tyr137Ile
Gly134Asp + Gly135Glu + Gly136Gln + Tyr137Thr
Leu133Gln + Gly134Glu + Gly135Asp + Tyr137Thr
Gly134Ser + Tyr137Val + Ser138Glu + Ser139Glu
Gly134Ser + Tyr137Val + Ser138Asp + Ser139Glu
Leu133His + Gly135Gln + Ser138Asp + Ser139Asp
Leu133Gly + Gly135Pro + Ser138Glu + Ser139Asp
Leu133Val + Gly135Pro + Ser138Asp + Ser139Glu
Leu133Ser + Gly134Gln + Gly135Asp + Gly136Glu
Leu133Ile + Gly134Gln + Ser139Asp + Ser140Asp
Leu133Cys + Tyr137Asn + Ser139Asp + Ser140Glu
Leu133Ser + Tyr137His + Ser139Glu + Ser140Glu
Gly134Pro + Gly136Gln + Ser139Asp + Ser140Glu
Gly134Gln + Gly135Ser + Ser139Asp + Ser140Asp
Leu133Cys + Gly134Pro + Ser139Glu + Ser140Asp
Leu133Ala + Gly136Asn + Tyr137Glu + Ser138Asp
Gly136Pro + Ser138Asp + Ser139Glu + Ser140Asp
Tyr137Leu + Ser138Asp + Ser139Glu + Ser140Glu
Gly135Asn + Ser138Asp + Ser139Asp + Ser140Glu
Leu133Gly + Ser138Asp + Ser139Glu + Ser140Glu

Leu133Ser	+ Tyr137Asp	+ Ser138Asp	+ Ser139Glu
Gly136Gln	+ Tyr137Asp	+ Ser138Asp	+ Ser139Asp
Gly136Asn	+ Tyr137Asp	+ Ser138Asp	+ Ser139Glu
Gly134Glu	+ Gly135Asp	+ Gly136Asp	+ Tyr137Ile
Leu133Gln	+ Tyr137Val	+ Ser138Glu	+ Ser140Asp
Gly134Pro	+ Tyr137Gly	+ Ser138Asp	+ Ser140Asp
Leu133His	+ Tyr137Leu	+ Ser138Glu	+ Ser140Glu
Gly134Gln	+ Gly135Ser	+ Ser138Glu	+ Ser140Glu
Leu133Cys	+ Gly136Ser	+ Ser138Glu	+ Ser140Glu
Gly135Gln	+ Gly136Gln	+ Ser138Asp	+ Ser140Asp
Gly134Pro	+ Tyr137His	+ Ser138Asp	+ Ser140Glu
Leu133Gln	+ Gly134Pro	+ Gly136Glu	+ Ser138Asp
Leu133His	+ Gly134Pro	+ Gly136Asp	+ Ser138Glu
Leu133Ile	+ Gly135Glu	+ Tyr137Glu	+ Ser138Glu
Gly134Asn	+ Gly135Asp	+ Tyr137Asp	+ Ser138Glu
Gly135Gln	+ Tyr137Glu	+ Ser138Asp	+ Ser140Glu
Gly136Asn	+ Tyr137Glu	+ Ser138Asp	+ Ser140Glu
Leu133Asn	+ Tyr137Asp	+ Ser138Glu	+ Ser140Asp
Leu133Cys	+ Gly136Pro	+ Tyr137Glu	+ Ser139Asp
Gly134Glu	+ Gly135Pro	+ Gly136Asp	+ Tyr137Glu
Gly136Pro	+ Tyr137Glu	+ Ser139Asp	+ Ser140Asp
Gly135Ser	+ Tyr137Glu	+ Ser139Glu	+ Ser140Glu
Leu133Pro	+ Gly134Glu	+ Gly136Asp	+ Tyr137Cys
Gly134Gln	+ Gly136Asp	+ Ser138Glu	+ Ser139Asp
Gly135Ser	+ Gly136Glu	+ Ser138Glu	+ Ser139Asp
Gly134Asp	+ Gly135Glu	+ Gly136Ser	- Ser138Asp

TABLE 14

Loop 4 - Single Mutation Variants
Gly160Asn
Gly160Asp
Gly160Gln
Gly160Glu
Gly160Pro
Gly160Ser
Asn161Asp
Asn161Gln
Asn161Glu
Asn161Ser
Asn162Asp
Asn162Gln
Asn162Glu
Asn162Ser
Asn163Asp
Asn163Gln
Asn163Glu
Asn163Ser
Ala164Asn
Ala164Asp
Ala164Gln
Ala164Glu

Ala164Gly
Ala164His
Ala164Pro
Ala164Ser
Ala164Thr
Asp165Glu
Ala166Asn
Ala166Asp
Ala166Gln
Ala166Glu
Ala166Gly
Ala166His
Ala166Pro
Ala166Ser
Ala166Thr
Arg167Asp
Arg167Glu
Asn168Asp
Asn168Gln
Asn168Glu
Asn168Ser
Tyr169Ala
Tyr169Asn
Tyr169Asp
Tyr169Cys
Tyr169Gln
Tyr169Glu
Tyr169Gly
Tyr169His
Tyr169Ile
Tyr169Leu
Tyr169Met
Tyr169Pro
Tyr169Ser
Tyr169Thr
Tyr169Val
Ser170Asp
Ser170Glu

TABLE 15

Loop 4 - Double Mutation Variants
Gly160Gln + Asp165Glu
Asn161Ser + Asn162Ser
Ala164Thr + Asn168Asp
Gly160Asn + Ser170Glu
Asn161Gln + Asn163Glu
Asn162Ser + Ser170Asp
Gly160Gln + Asn168Glu
Ala166Gly + Ser170Asp
Asn161Glu + Asn162Ser
Asn162Gln + Ser170Asp

Ala166Thr + Ser170Glu
Asn162Ser + Ala164Pro
Gly160Asn + Asn161Gln
Gly160Glu + Ala166Ser
Ala166Gly + Ser170Glu
Ala164His + Ala166Gly
Ala164Asn + Asp165Glu
Asn162Asp + Asn168Ser
Ala164Pro + Asn168Asp
Asn162Asp + Ala164Asn
Tyr169Thr + Ser170Asp
Ala164Glu + Tyr169His
Asp165Glu + Tyr169His
Asn162Glu + Asn168Ser
Gly160Asp + Tyr169Met
Asn162Glu + Ala166Asn
Asn161Glu + Ala166Thr
Asp165Glu + Ala166Asn
Asn161Gln + Tyr169Pro
Asn162Asp + Ala164Thr
Asn162Ser + Arg167Glu
Ala166Pro + Arg167Asp
Ala164Thr + Ser170Asp
Asn163Asp + Ala164Asn
Asp165Glu + Asn168Gln
Asn163Asp + Tyr169Thr
Asn168Asp + Tyr169Gly
Arg167Asp + Asn168Ser
Asn161Ser + Arg167Glu
Asn161Ser + Ser170Glu
Gly160Asp + Ala166Gly
Ala164Asn + Ala166Gln
Gly160Asn + Asn161Glu
Asn162Glu + Ala166Ser
Ala166His + Asn168Asp
Gly160Pro + Ala164Gly
Gly160Asn + Ala164Gly
Ala164Gln + Ser170Asp
Asn161Glu + Ala164Thr
Gly160Glu + Ala166Gly
Ala166Pro + Ser170Asp
Ala166Pro + Tyr169Ala
Gly160Ser + Arg167Glu
Gly160Asp + Ala164Ser
Asn161Gln + Ala164Pro
Asn163Glu + Ala166Gln
Ala164Ser + Asn168Gln
Gly160Glu + Ala164Ser
Gly160Asp + Ala166Gln
Ala164Thr + Asp165Glu

TABLE 16

Loop 4 - Triple Mutation Variants

Gly160Gln	+ Asn161Ser	+ Asp165Glu
Ala166Gly	+ Arg167Glu	+ Tyr169Cys
Asn162Ser	+ Ala166Asn	+ Ser170Asp
Gly160Gln	+ Asn162Asp	+ Asn168Ser
Ala164Asn	+ Asn168Gln	+ Ser170Glu
Ala164Asp	+ Ala166Thr	+ Asn168Ser
Asn163Gln	+ Ala166Gly	+ Arg167Glu
Asn162Ser	+ Ala166His	+ Asn168Gln
Asp165Glu	+ Ala166Thr	+ Asn168Gln
Asn163Ser	+ Ala164Pro	+ Asn168Asp
Gly160Glu	+ Ala164His	+ Tyr169Gln
Asn161Gln	+ Asn163Ser	+ Ser170Glu
Ala166Thr	+ Tyr169Leu	+ Ser170Asp
Gly160Ser	+ Ala164Asn	+ Asn168Glu
Asn161Gln	+ Asn162Glu	+ Asn163Ser
Asn163Ser	+ Ala166Ser	+ Arg167Glu
Ala164Thr	+ Ala166Gly	+ Arg167Glu
Ala164Ser	+ Ala166Gly	+ Ser170Glu
Asn162Gln	+ Ala164Gln	+ Ala166Pro
Asn162Glu	+ Asn163Gln	+ Ala164Gly
Asn161Asp	+ Ala164Asn	+ Tyr169Val
Gly160Asn	+ Ala164Glu	+ Ala166Pro
Asn162Gln	+ Ala164Pro	+ Arg167Asp
Asn163Ser	+ Ala164Ser	+ Tyr169Gly
Ala166Asn	+ Arg167Glu	+ Asn168Ser
Asn163Gln	+ Asp165Glu	+ Ala166Ser
Asn162Ser	+ Ala164His	+ Asn166Asp
Ala164Ser	+ Arg167Asp	+ Asn166Asp
Asn162Ser	+ Arg167Asp	+ Asn168Asp
Asn161Asp	+ Asn162Glu	+ Asn163Gln
Asn161Asp	+ Asn162Glu	+ Asn168Gln
Gly160Glu	+ Asn161Glu	+ Ala166Asn
Gly160Glu	+ Asn161Glu	+ Ala164Ser
Gly160Pro	+ Asn162Asp	+ Asn163Asp
Asn162Asp	+ Asn163Glu	+ Ala166Asn
Asn161Glu	+ Asn162Asp	+ Asn163Glu
Asn162Glu	+ Asn163Ser	+ Ala164Asp
Asp165Glu	+ Arg167Asp	+ Tyr169Pro
Asp165Glu	+ Arg167Asp	+ Asn168Asp
Ala164Gln	+ Arg167Asp	+ Tyr169Asp
Asn161Asp	+ Asn162Asp	+ Ala164Asp
Asn161Asp	+ Asn163Asp	+ Tyr169Thr
Asn161Asp	+ Asn163Asp	+ Ala164Asn
Gly160Glu	+ Asn162Glu	+ Asn163Gln
Ala164Asp	+ Asp165Glu	+ Arg167Glu
Ala164Pro	+ Asp165Glu	+ Asn168Asp
Gly160Asp	+ Asn162Glu	+ Ala164Glu
Gly160Pro	+ Asn168Glu	+ Ser170Glu

Ala164Glu + Arg167Glu + Asn168Glu
Asn161Glu + Asn163Glu + Asp165Glu
Gly160Asp + Asn162Glu + Asp165Glu
Asp165Glu + Ala166Gly + Tyr169Glu
Gly160Glu + Asn161Asp + Asp165Glu
Arg167Asp + Asn168Gln + Ser170Asp
Asn162Gln + Arg167Asp + Ser170Glu
Asn163Gln + Arg167Glu + Ser170Glu
Asn162Asp + Asp165Glu + Asn168Glu
Asn162Asp + Asp165Glu + Arg167Glu
Gly160Asp + Asn168Gln + Ser170Glu
Gly160Asp + Asp165Glu + Tyr169Gly

TABLE 17

Loop 4 - Quadruple Mutation Variants

Gly160Gln + Asn161Ser + Asn162Ser + Asp165Glu
Gly160Glu + Asn161Ser + Asn162Gln + Asn168Ser
Asn161Ser + Asn163Asp + Ala164Asn + Tyr169Thr
Gly160Ser + Asp165Glu + Ala166Asn + Tyr169Leu
Gly160Gln + Ala166Ser + Arg167Asp + Tyr169His
Gly160Ser + Asn163Ser + Ala166Gly + Ser170Asp
Asn161Gln + Asn163Gln + Ala164Glu + Ala166Gly
Asn161Glu + Asn163Gln + Ala166His + Tyr169Asn
Asn163Gln + Ala166His + Arg167Asp + Asn168Gln
Gly160Gln + Asn162Ser + Asn163Asp + Tyr169Gln
Asn162Glu + Ala166Gln + Asn168Ser + Tyr169Gly
Asn163Glu + Ala166His + Asn168Gln + Tyr169Gln
Gly160Asn + Asn161Ser + Ala164Thr + Ala166Ser
Asn161Gln + Asn162Ser + Asn168Glu + Tyr169Ser
Asn161Gln + Ala166His + Arg167Glu + Tyr169Ala
Asn161Gln + Ala164Gly + Ala166Gln + Tyr169His
Ala164Gln + Asp165Glu + Asn168Ser + Tyr169Met
Ala164Gln + Ala166His + Arg167Glu + Asn168Glu
Gly160Asn + Asn162Ser + Ala164Glu + Asp165Glu
Asn161Asp + Asn162Asp + Ala164Asn + Ala166Ser
Asn161Asp + Asn162Asp + Ala164Asn + Ala166Thr
Ala164Gln + Ala166Asn + Asn168Asp + Tyr169Asp
Gly160Pro + Asn163Asp + Ala164Asp + Ala166Thr
Asn161Ser + Asn163Asp + Ala164Asp + Asn168Gln
Asn162Asp + Asn163Asp + Ala166Pro + Tyr169Met
Asn161Ser + Asn163Gln + Tyr169Asp + Ser170Asp
Asn161Ser + Ala166His + Tyr169Asp + Ser170Glu
Asn161Glu + Asn162Asp + Asn163Glu + Tyr169Cys
Asn161Glu + Asn162Glu + Asn163Asp + Tyr169Ala
Asp165Glu + Ala166Gly + Arg167Asp + Asn168Gln
Asp165Glu + Arg167Asp + Asn168Gln + Tyr169Val
Asn161Asp + Asn163Glu + Ala164Asn + Ala166Gly
Asn163Gln + Ala164Asp + Asp165Glu + Arg167Asp
Asp165Glu + Ala166Asn + Asn168Asp + Tyr169Glu
Asn163Gln + Asp165Glu + Arg167Glu + Tyr169Asp
Asp165Glu + Arg167Asp + Asn168Ser + Tyr169Asp

Asn163Ser	+ Asp165Glu	+ Arg167Asp	+ Tyr169Glu
Asn163Gln	+ Ala166Thr	+ Asn168Glu	+ Ser170Glu
Asn161Gln	+ Ala166Gly	+ Asn168Asp	+ Ser170Glu
Asn163Glu	+ Asp165Glu	+ Ala166Gly	+ Tyr169Leu
Gly160Pro	+ Asn162Gln	+ Asn163Asp	+ Asp165Glu
Gly160Glu	+ Asn163Gln	+ Asn168Asp	+ Tyr169Glu
Gly160Asp	+ Ala164Glu	+ Asp165Glu	+ Tyr169Ser
Asn161Glu	+ Ala164Asp	+ Asp165Glu	+ Tyr169Gln
Gly160Asp	+ Asn162Asp	+ Ala166Gly	+ Tyr169Asp
Asn161Glu	+ Asn162Glu	+ Asp165Glu	+ Ala166Asn
Asn161Asp	+ Asn162Asp	+ Asp165Glu	+ Ala166Gln
Asn161Asp	+ Asn163Asp	+ Asp165Glu	+ Asn168Gln
Gly160Asp	+ Asn161Ser	+ Asn163Glu	+ Tyr169Gly
Gly160Asp	+ Asn162Asp	+ Asp165Glu	+ Asn168Gln
Asn161Ser	+ Arg167Glu	+ Tyr169Thr	+ Ser170Asp
Asn161Gln	+ Arg167Glu	+ Asn168Gln	+ Ser170Asp
Gly160Asp	+ Asn163Asp	+ Asp165Glu	+ Tyr169Ala
Gly160Ser	+ Asn162Glu	+ Ala164Pro	+ Asp165Glu
Gly160Glu	+ Arg167Glu	+ Asn168Glu	+ Tyr169Gln
Asn161Ser	+ Ala164Asp	+ Arg167Glu	+ Tyr169Asn
Asn162Ser	+ Asn163Asp	+ Ala164Asp	+ Asn168Asp
Asn163Glu	+ Asp165Glu	+ Ala166Asn	+ Arg167Glu
Asn163Asp	+ Asp165Glu	+ Arg167Asp	+ Asn168Ser
Gly160Glu	+ Ala164Gly	+ Asn168Asp	+ Ser170Asp

TABLE 18

Loop 5 - Single Mutation Variants

Ser190Asp
Ser190Glu
Ser191Asp
Ser191Glu
Phe192Ala
Phe192Asn
Phe192Asp
Phe192Cys
Phe192Gln
Phe192Glu
Phe192Gly
Phe192His
Phe192Ile
Phe192Leu
Phe192Met
Phe192Pro
Phe192Ser
Phe192Thr
Phe192Tyr
Phe192Val
Ser193Asp
Ser193Glu
Asn194Asp
Asn194Gln

Asn194Glu
Asn194Ser

TABLE 19

Loop 5 - Double Mutation Variants

Ser190Asp + Phe192Leu
Ser190Glu + Phe192Gln
Phe192Glu + Asn194Gln
Phe192Tyr + Asn194Glu
Ser190Asp + Phe192Ile
Ser191Glu + Phe192Ser
Ser191Asp + Phe192Gly
Ser191Asp + Asn194Ser
Ser191Glu + Phe192Thr
Ser191Glu + Asn194Gln
Ser191Glu + Phe192His
Ser191Asp + Phe192Asn
Ser190Asp + Phe192Gly
Ser191Asp + Asn194Gln
Ser191Asp + Phe192Ala
Ser190Glu + Phe192Cys
Ser190Glu + Phe192Ile
Ser191Asp + Phe192Ser
Ser190Asp + Asn194Gln
Ser191Glu + Phe192Cys
Phe192Ser + Asn194Glu
Ser191Glu + Phe192Gly
Phe192Gly + Asn194Gln
Phe192Cys + Asn194Gln
Phe192Asn + Asn194Asp
Ser190Glu + Phe192Tyr
Ser191Glu + Phe192Ala
Ser190Glu + Asn194Ser
Phe192Ile + Asn194Ser
Ser191Glu + Phe192Met
Ser190Asp + Phe192Met
Ser190Asp + Asn194Ser
Phe192Gly + Asn194Asp
Phe192His + Asn194Ser
Ser190Glu + Asn194Gln
Phe192Ala + Asn194Glu
Phe192Thr + Asn194Glu
Ser190Glu + Phe192Ser
Ser191Glu + Phe192Ile
Phe192Val + Asn194Glu
Phe192Thr + Asn194Asp
Ser191Glu + Phe192Pro
Ser191Asp + Phe192Leu
Phe192Ile + Asn194Glu
Ser190Asp + Phe192Thr
Ser191Asp + Phe192Ile

Phe192Thr + Asn194Gln
Phe192Ala + Asn194Gln
Ser191Asp + Phe192Tyr
Ser190Glu + Phe192Thr
Phe192Leu + Asn194Glu
Phe192His + Asn194Gln
Ser190Asp + Phe192Tyr
Phe192Val + Asn194Ser
Phe192Asp + Asn194Gln
Phe192Gly + Asn194Ser
Ser191Asp + Phe192Gln
Phe192Asp + Asn194Ser
Phe192Thr + Asn194Ser
Ser190Glu + Phe192Ala

TABLE 20

Loop 5 - Triple Mutation Variants

Ser191Asp + Phe192Met + Asn194Gln
Ser191Glu + Phe192Ala + Asn194Gln
Ser191Asp + Phe192Leu + Asn194Ser
Ser191Glu + Phe192Asn + Asn194Gln
Ser190Asp + Phe192Gln + Asn194Gln
Ser191Asp + Phe192Ile + Asn194Gln
Ser190Asp + Phe192Asn + Asn194Ser
Ser191Glu + Phe192Ile + Asn194Ser
Ser190Asp + Phe192Leu + Asn194Gln
Ser190Asp + Phe192Val + Asn194Ser
Ser190Glu + Phe192Gln + Asn194Ser
Ser191Asp + Phe192Val + Asn194Ser
Ser191Glu + Phe192Ala + Asn194Ser
Ser191Glu + Phe192Cys + Asn194Gln
Ser190Glu + Phe192Val + Asn194Ser
Ser190Glu + Phe192Ile + Asn194Gln
Ser190Glu + Phe192Ser + Asn194Ser
Ser190Glu + Phe192Tyr + Asn194Gln
Ser191Asp + Phe192Ile + Asn194Ser
Ser190Asp + Ser191Glu + Asn194Ser
Ser190Asp + Ser191Asp + Phe192Met
Ser190Glu + Ser191Glu + Phe192Val
Ser190Asp + Ser191Glu + Phe192Ser
Ser190Asp + Ser191Asp + Phe192Thr
Ser190Glu + Ser191Asp + Asn194Ser
Ser190Glu + Ser191Asp + Phe192Asn
Ser190Asp + Ser191Asp + Phe192Asn
Ser190Asp + Ser191Asp + Phe192Val
Ser190Glu + Ser191Glu + Phe192Cys
Ser190Asp + Ser191Glu + Phe192Asn
Ser190Glu + Ser191Glu + Phe192Leu
Ser190Glu + Ser191Asp + Phe192Gly
Ser190Glu + Ser191Glu + Phe192Ser
Ser190Glu + Ser191Glu + Asn194Ser

Ser190Asp + Ser191Asp + Asn194Gln
Ser190Asp + Ser191Asp + Phe192Tyr
Ser190Asp + Ser191Glu + Phe192Met
Ser190Asp + Ser191Asp + Phe192Gly
Ser190Glu + Ser191Asp + Asn194Gln
Ser190Asp + Ser191Glu + Phe192Leu
Ser190Asp + Ser191Glu + Phe192Gly
Ser190Glu + Ser191Asp + Phe192Tyr
Ser190Asp + Ser191Glu + Phe192Thr
Ser190Glu + Ser191Asp + Phe192His
Ser190Asp + Ser191Asp + Phe192Gln
Ser190Glu + Ser191Asp + Phe192Ile
Ser190Asp + Ser191Glu + Asn194Gln
Ser190Asp + Ser191Asp + Phe192Pro
Ser190Glu + Ser191Asp + Phe192Cys
Ser190Asp + Ser191Asp + Asn194Ser
Ser190Asp + Ser191Glu + Phe192Gln
Ser190Glu + Ser191Asp + Phe192Pro
Ser191Asp + Phe192Asp + Asn194Gln
Ser191Asp + Phe192Glu + Asn194Ser
Ser191Asp + Phe192Glu + Asn194Gln
Ser190Asp + Ser191Glu + Phe192Asp
Ser190Asp + Ser191Asp + Phe192Glu
Ser190Glu + Ser191Glu + Phe192Asp
Ser190Glu + Ser191Asp + Phe192Asp
Ser190Glu + Ser191Asp + Phe192Glu

TABLE 21

Loop 5 - Quadruple Mutation Variants

Ser190Glu + Ser191Asp + Phe192Ile + Asn194Ser
Ser190Asp + Ser191Asp + Phe192Gly + Asn194Gln
Ser190Glu + Ser191Asp + Phe192His + Asn194Ser
Ser190Glu + Ser191Asp + Phe192Gln + Asn194Gln
Ser190Asp + Ser191Asp + Phe192Met + Asn194Ser
Ser190Asp + Ser191Glu + Phe192Leu + Asn194Gln
Ser190Glu + Ser191Glu + Phe192Ala + Asn194Ser
Ser190Asp + Ser191Asp + Phe192Pro + Asn194Gln
Ser190Glu + Ser191Glu + Phe192Leu + Asn194Ser
Ser190Glu + Ser191Asp + Phe192His + Asn194Gln
Ser190Glu + Ser191Asp + Phe192Cys + Asn194Gln
Ser190Glu + Ser191Asp + Phe192Thr + Asn194Ser
Ser190Glu + Ser191Asp + Phe192Ser + Asn194Gln
Ser190Glu + Ser191Asp + Phe192Thr + Asn194Gln
Ser190Asp + Ser191Glu + Phe192Ile + Asn194Ser
Ser190Glu + Ser191Asp + Phe192Gln + Asn194Ser
Ser190Asp + Ser191Asp + Phe192Cys + Asn194Gln
Ser190Asp + Ser191Asp + Phe192Pro + Asn194Ser
Ser190Glu + Ser191Glu + Phe192Ala + Asn194Gln
Ser190Asp + Ser191Glu + Phe192Thr + Asn194Ser
Ser190Glu + Ser191Asp + Phe192Leu + Asn194Gln
Ser190Glu + Ser191Asp + Phe192Gly + Asn194Gln

Ser190Asp	+ Ser191Asp	+ Phe192Cys	+ Asn194Ser
Ser190Glu	+ Ser191Glu	+ Phe192His	+ Asn194Gln
Ser190Glu	+ Ser191Asp	+ Phe192Asn	+ Asn194Ser
Ser190Asp	+ Ser191Asp	+ Phe192His	+ Asn194Gln
Ser190Glu	+ Ser191Glu	+ Phe192Ile	+ Asn194Gln
Ser190Asp	+ Ser191Asp	+ Phe192Tyr	+ Asn194Ser
Ser190Glu	+ Ser191Asp	+ Phe192Tyr	+ Asn194Gln
Ser190Glu	+ Ser191Asp	+ Phe192Ala	+ Asn194Ser
Ser190Asp	+ Ser191Glu	+ Phe192Ala	+ Asn194Ser
Ser190Asp	+ Ser191Glu	+ Phe192Asn	+ Asn194Ser
Ser190Glu	+ Ser191Asp	+ Phe192Val	+ Asn194Ser
Ser190Asp	+ Ser191Asp	+ Phe192Gln	+ Asn194Ser
Ser190Asp	+ Ser191Asp	+ Phe192Gln	+ Asn194Gln
Ser190Asp	+ Ser191Asp	+ Phe192Leu	+ Asn194Gln
Ser190Asp	+ Ser191Asp	+ Phe192Ser	+ Asn194Gln
Ser190Glu	+ Ser191Asp	+ Phe192Glu	+ Asn194Ser
Ser190Asp	+ Ser191Asp	+ Phe192Asp	+ Asn194Ser
Ser190Glu	+ Ser191Asp	+ Phe192Glu	+ Asn194Gln
Ser190Glu	+ Ser191Glu	+ Phe192Glu	+ Asn194Ser
Ser190Asp	+ Ser191Glu	+ Phe192Glu	+ Asn194Gln
Ser190Asp	+ Ser191Asp	+ Phe192Glu	+ Asn194Ser
Ser190Glu	+ Ser191Glu	+ Phe192Asp	+ Asn194Ser
Ser190Glu	+ Ser191Asp	+ Phe192Asp	+ Asn194Ser
Ser190Glu	+ Ser191Glu	+ Phe192Asp	+ Asn194Gln
Ser190Asp	+ Ser191Asp	+ Phe192Asp	+ Asn194Gln
Ser190Asp	+ Ser191Glu	+ Phe192Glu	+ Asn194Ser
Ser190Glu	+ Ser191Glu	+ Phe192Tyr	+ Asn194Glu
Ser190Glu	+ Ser191Glu	+ Phe192Met	+ Asn194Glu
Ser190Asp	+ Ser191Glu	+ Phe192Gln	+ Asn194Asp
Ser190Asp	+ Ser191Asp	+ Phe192Ala	+ Asn194Asp
Ser190Glu	+ Ser191Glu	+ Phe192Leu	+ Asn194Asp
Ser190Glu	+ Ser191Glu	+ Phe192Ser	+ Asn194Asp
Ser190Glu	+ Ser191Glu	+ Phe192Met	+ Asn194Asp
Ser190Asp	+ Ser191Glu	+ Phe192Tyr	+ Asn194Glu
Ser190Asp	+ Ser191Asp	+ Phe192His	+ Asn194Asp
Ser190Glu	+ Ser191Asp	+ Phe192Gln	+ Asn194Asp
Ser190Asp	+ Ser191Asp	+ Phe192Asn	+ Asn194Glu
Ser190Asp	+ Ser191Glu	+ Phe192Ile	+ Asn194Asp

TABLE 22

Loop 6 - Single Mutation Variants

Gly203Asn
Gly203Asp
Gly203Gln
Gly203Glu
Gly203Pro
Gly203Ser
Pro204Asn
Pro204Asp
Pro204Gln
Pro204Glu

Pro204Gly
Pro204Ser
Gly205Asn
Gly205Asp
Gly205Gln
Gly205Glu
Gly205Pro
Gly205Ser
Thr206Asn
Thr206Asp
Thr206Gln
Thr206Glu
Thr206Gly
Thr206Pro
Thr206Ser
Ser207Asp
Ser207Glu
Ile208Ala
Ile208Asn
Ile208Asp
Ile208Cys
Ile208Gln
Ile208Glu
Ile208Gly
Ile208His
Ile208Leu
Ile208Met
Ile208Pro
Ile208Ser
Ile208Thr
Ile208Val
Leu209Ala
Leu209Asn
Leu209Asp
Leu209Cys
Leu209Gln
Leu209Glu
Leu209Gly
Leu209His
Leu209Ile
Leu209Met
Leu209Pro
Leu209Ser
Leu209Thr
Leu209Val
Ser210Asp
Ser210Glu
Thr211Asn
Thr211Asp
Thr211Gln
Thr211Glu
Thr211Gly
Thr211Pro

Thr211Ser
Trp212Ala
Trp212Asn
Trp212Asp
Trp212Cys
Trp212Gln
Trp212Glu
Trp212Gly
Trp212His
Trp212Ile
Trp212Leu
Trp212Met
Trp212Phe
Trp212Pro
Trp212Ser
Trp212Thr
Trp212Tyr
Trp212Val
Ile213Ala
Ile213Asn
Ile213Asp
Ile213Cys
Ile213Gln
Ile213Glu
Ile213Gly
Ile213His
Ile213Leu
Ile213Met
Ile213Pro
Ile213Ser
Ile213Thr
Ile213Val
Gly214Asn
Gly214Asp
Gly214Gln
Gly214Glu
Gly214Pro
Gly214Ser
Gly215Asn
Gly215Asp
Gly215Gln
Gly215Glu
Gly215Pro
Gly215Ser
Ser216Asp
Ser216Glu
Thr217Asn
Thr217Asp
Thr217Gln
Thr217Glu
Thr217Gly
Thr217Pro
Thr217Ser

Arg218Asp
Arg218Glu
Ser219Asp
Ser219Glu
Ile220Ala
Ile220Asn
Ile220Asp
Ile220Cys
Ile220Gln
Ile220Glu
Ile220Gly
Ile220His
Ile220Leu
Ile220Met
Ile220Pro
Ile220Ser
Ile220Thr
Ile220Val
Ser221Asp
Ser221Glu
Gly222Asn
Gly222Asp
Gly222Gln
Gly222Glu
Gly222Pro
Gly222Ser
Thr223Asn
Thr223Asp
Thr223Gln
Thr223Glu
Thr223Gly
Thr223Pro
Thr223Ser

TABLE 23

Loop 6 - Double Mutation Variants
Gly203Gln + Ser219Glu
Thr206Gly + Leu209Ser
Trp212Phe + Ser221Glu
Arg218Glu + Gly222Pro
Gly214Asp + Ile220Ser
Gly215Asp + Thr217Gly
Gly203Asn + Ser219Asp
Trp212Ile + Ser221Glu
Trp212Gln + Ser219Asp
Trp212Cys + Ser219Asp
Thr217Glu + Thr223Pro
Gly214Pro + Ile220Val
Ile208Pro + Thr223Ser
Leu209Gln + Ile220Glu
Thr206Asn + Ile220His

Ile208Ala + Thr217Ser
Pro204Gln + Ser221Asp
Trp212Thr + Thr223Asn
Gly203Asn + Ser219Glu
Gly203Gln + Gly214Pro
Gly214Asp + Gly215Gln
Gly203Ser + Arg218Glu
Thr206Gln + Trp212Asn
Pro204Ser + Ile213Glu
Ile208Leu + Gly222Glu
Gly215Gln + Thr217Gln
Ile208Thr + Ser219Asp
Gly205Ser + Gly214Asp
Ile208Ser + Arg218Glu
Ile208Ser + Ile220Val
Thr211Gly + Arg218Asp
Ile213Asp + Ile220Ser
Pro204Gln + Ser219Glu
Thr211Gly + Ile220Met
Ile208His + Leu209Ala
Gly205Asn + Ser207Glu
Thr206Ser + Gly215Asn
Gly205Asn + Gly222Ser
Gly203Pro + Ile213Cys
Thr206Gln + Gly215Ser
Ile208Ser + Gly214Glu
Thr217Gln + Ser219Glu
Pro204Ser + Ser216Asp
Gly214Asn + Ser216Asp
Ile208Leu + Thr211Asn
Pro204Gly + Ser207Glu
Ser219Asp + Gly222Asn
Trp212Tyr + Ile213Thr
Thr206Ser + Trp212Val
Ile220Asp + Thr223Pro
Ile208Pro + Trp212Pro
Ile208Leu + Thr217Asp
Pro204Ser + Gly214Ser
Gly203Ser + Gly222Pro
Gly205Ser + Arg218Glu
Gly203Pro + Ser207Asp
Ile208Val + Ser219Glu
Ser216Asp + Gly222Gln
Ser207Asp + Trp212Ala
Ile220Cys + Gly222Glu
Thr206Asn + Ile213Cys
Thr206Asp + Gly215Gln
Ser207Asp + Thr211Ser
Ile208Leu + Ser216Glu
Leu209Val + Gly214Asp
Thr206Glu + Ile213Asn
Pro204Ser + Thr211Ser
Ile213Asp + Gly215Pro

Gly214Asp + Thr223Ser
Gly205Asn + Leu209Cys
Gly205Gln + Ile208Ala
Ile208Leu + Ser219Asp
Leu209Glu + Thr211Pro
Ile208Cys + Thr217Gln
Pro204Asn + Leu209Asn
Gly214Pro + Ile220Pro
Gly203Gln + Thr211Gly
Pro204Gly + Thr223Asp
Gly215Ser + Thr217Pro
Thr206Gln + Thr211Asn
Gly205Pro + Gly215Pro
Gly203Pro + Ser207Glu
Ser207Glu + Ile208His
Pro204Ser + Thr223Gly
Gly205Asn + Gly215Asp
Leu209Gln + Ser219Glu
Gly215Pro + Thr223Ser
Ile208Gln + Ser221Asp
Gly203Gln + Arg218Asp
Gly203Asn + Ile220Asp
Gly205Pro + Arg218Asp
Thr211Asn + Arg218Glu
Gly205Asn + Thr223Glu
Thr206Glu + Leu209His
Thr206Pro + Gly215Asp
Trp212Gln + Ser221Asp
Ser216Glu + Ile220Met
Gly215Asn + Gly222Asp
Ile213Cys + Ser219Asp
Pro204Ser + Thr211Asn
Pro204Ser + Thr223Pro
Pro204Gln + Ile213Glu
Gly214Ser + Ser221Asp
Ile208Leu + Thr223Pro
Ser207Asp + Ile208Gly
Ile208Leu + Arg218Glu
Leu209Ser + Ile220His
Ile213Leu + Arg218Asp
Pro204Ser + Ser219Asp
Gly203Ser + Gly222Glu
Gly205Pro + Thr223Asn
Trp212Thr + Gly214Ser
Thr206Ser + Ser216Asp
Gly203Pro + Thr206Gly
Thr211Pro + Gly222Ser
Ile213Thr + Thr223Glu
Thr206Pro + Ile213Glu
Ser207Asp + Gly222Asn
Ile213Val + Gly214Asn
Thr211Asn + Thr223Asn
Ile213Cys + Thr223Pro

Pro204Ser + Gly222Gln
Gly205Gln + Gly215Glu
Leu209Pro + Gly222Gln
Gly205Ser + Gly222Asp
Pro204Ser + Ile208Leu
Pro204Gln + Thr211Gly
Leu209Ser + Ser219Asp
Thr217Glu + Thr223Asn
Gly203Gln + Thr217Ser
Gly205Pro + Ile213Gln
Leu209Glu + Thr217Ser
Ser216Asp + Thr217Ser
Trp212Gln + Ile213Ala
Arg218Glu + Ile220Gln
Leu209Ile + Thr223Asn
Ile208Met + Gly215Gln
Arg218Asp + Ile220Ala
Gly203Asn + Pro204Asn
Ile208His + Ser221Asp
Ile213Asn + Ser219Asp
Trp212Met + Ser219Glu
Thr206Pro + Ser216Glu
Pro204Gln + Thr206Asp
Thr206Gly + Gly215Pro
Pro204Asn + Gly222Gln
Ser219Glu + Gly222Ser
Ile220Asn + Gly222Asn
Gly205Asn + Ile213Asn
Gly203Ser + Ile213Leu
Ser207Asp + Thr211Asn
Leu209Pro + Ile213Glu
Ile208Pro + Thr223Asp
Thr211Ser + Gly222Asp
Thr206Ser + Thr217Asp
Gly203Gln + Gly214Glu
Gly205Ser + Gly215Ser
Gly203Gln + Leu209Ala
Gly203Gln + Gly222Asn
Ser207Asp + Thr211Gly
Gly205Gln + Ser219Glu
Trp212Cys + Ser216Asp
Gly203Gln + Thr217Glu
Arg218Glu + Ile220Cys
Thr211Gly + Ser221Glu
Pro204Asn + Ser216Asp
Arg218Asp + Gly222Pro
Gly205Pro + Ser221Glu
Arg218Glu + Thr223Ser
Leu209Asn + Ser216Glu
Gly205Pro + Ile220Met
Thr206Asn + Thr217Asp
Gly203Ser + Ile208Thr
Thr217Ser + Arg218Glu

Pro204Asn	+	Thr211Asn
Gly205Asn	+	Thr217Glu
Ser207Asp	+	Thr223Gly
Trp212Tyr	+	Ile213Pro
Gly214Gln	+	Ser216Asp
Thr206Asp	+	Ile208Thr

TABLE 24

Loop 6 - Triple Mutation Variants

Gly203Gln	+	Thr206Gly	+	Ser219Glu
Ile213Ala	+	Gly215Asp	+	Thr217Gly
Gly203Asn	+	Ser219Asp	+	Thr223Ser
Gly215Pro	+	Ser216Glu	+	Thr223Ser
Gly203Gln	+	Trp212Pro	+	Gly222Asp
Pro204Ser	+	Gly205Ser	+	Ser207Glu
Gly205Pro	+	Ile208Met	+	Ser219Asp
Thr211Gly	+	Gly215Gln	+	Thr217Asn
Thr211Ser	+	Thr217Gln	+	Thr223Asp
Gly205Gln	+	Thr211Asn	+	Gly222Asn
Thr211Pro	+	Gly214Asn	+	Thr217Asn
Gly205Pro	+	Leu209Asn	+	Thr223Gln
Gly203Gln	+	Trp212Leu	+	Ile220Ala
Trp212Thr	+	Gly215Glu	+	Ile220Thr
Gly205Gln	+	Ser207Asp	+	Trp212Ala
Gly205Asn	+	Trp212Phe	+	Ser216Glu
Ser207Glu	+	Thr211Gly	+	Gly222Asn
Pro204Gln	+	Gly205Ser	+	Ser221Glu
Thr206Pro	+	Leu209Gly	+	Thr223Asn
Ile208Met	+	Thr217Gly	+	Thr223Gln
Thr211Ser	+	Gly215Pro	+	Gly222Glu
Ile208Pro	+	Trp212Pro	+	Ser216Glu
Gly203Ser	+	Thr211Gln	+	Gly222Pro
Gly203Pro	+	Ile208Val	+	Ser219Glu
Thr206Pro	+	Gly215Ser	+	Ser216Glu
Thr206Pro	+	Ile220Asn	+	Thr223Gly
Pro204Gln	+	Gly205Pro	+	Arg218Glu
Trp212His	+	Ile213Met	+	Gly215Asp
Ile208Ser	+	Gly222Gln	+	Thr223Gln
Gly205Gln	+	Trp212Leu	+	Arg218Asp
Gly214Gln	+	Ser216Glu	+	Gly222Ser
Gly203Asn	+	Ser216Glu	+	Gly222Gln
Leu209Asn	+	Gly222Glu	+	Thr223Ser
Thr206Asp	+	Thr211Asn	+	Gly214Ser
Pro204Asn	+	Thr211Gly	+	Trp212Thr
Gly203Ser	+	Ile208Ala	+	Ser216Glu
Thr211Ser	+	Gly214Pro	+	Gly222Glu
Gly215Gln	+	Arg218Asp	+	Ile220Val
Gly215Ser	+	Ser216Asp	+	Thr223Ser
Thr206Asn	+	Thr211Asn	+	Arg218Glu
Pro204Asn	+	Thr217Glu	+	Thr223Ser
Thr206Ser	+	Ile213Met	+	Gly215Ser

Pro204Gly + Gly205Ser + Trp212Gln
Thr211Pro + Trp212Ala + Thr223Asp
Gly215Glu + Ile220Ala + Thr223Gln
Thr206Gln + Ile208Ser + Leu209Asp
Thr211Ser + Trp212Val + Ser219Glu
Gly205Pro + Thr211Pro + Ser216Asp
Gly205Pro + Trp212His + Ser219Glu
Gly205Pro + Trp212Asn + Ser219Asp
Leu209Ser + Thr211Gln + Arg218Asp
Pro204Gly + Thr211Asn + Gly215Glu
Gly203Pro + Pro204Ser + Ser216Asp
Gly203Gln + Ile213Val + Arg218Glu
Ile208Gly + Thr217Asn + Gly222Ser
Pro204Ser + Gly205Gln + Trp212Ile
Pro204Gln + Gly214Glu + Gly215Gln
Trp212Cys + Gly215Gln + Ser221Glu
Gly203Asn + Leu209Val + Thr217Glu
Gly205Asn + Ser207Asp + Thr217Gly
Pro204Gln + Thr206Asp + Gly215Ser
Ile208Gln + Thr211Pro + Ser219Asp
Gly205Ser + Ile213His + Ser219Glu
Gly203Ser + Ile208Ser + Trp212Met
Thr211Pro + Trp212Tyr + Ser219Asp
Gly205Gln + Ile208His + Gly222Asn
Gly203Ser + Thr211Ser + Ile220Glu
Pro204Gly + Leu209Ile + Arg218Glu
Trp212Tyr + Ile213Ser + Thr217Pro
Ile208Asn + Ile213Pro + Gly214Ser
Gly203Gln + Ile213His + Ser219Asp
Gly205Asn + Thr211Gln + Ser221Glu
Gly205Asn + Ser207Glu + Gly214Gln
Ile208Val + Gly214Glu + Gly222Gln
Gly203Ser + Thr206Gly + Ile213Leu
Pro204Gly + Ile208Pro + Gly215Asp
Thr206Glu + Thr217Gly + Ile220Pro
Ile213Ser + Thr217Asp + Gly222Gln
Ile208Val + Gly215Asn + Thr223Ser
Gly205Pro + Ser207Glu + Thr217Ser
Gly203Pro + Thr206Glu + Ile208His
Gly205Ser + Trp212Cys + Ser216Asp
Pro204Ser + Thr206Asp + Leu209Ile
Thr206Glu + Ile208Thr + Ile220Gly
Ile213Gln + Ile220Glu + Gly222Gln
Ile208Val + Gly214Gln + Gly215Pro
Leu209Pro + Ser216Glu + Thr217Asn
Pro204Gln + Trp212Met + Gly222Glu
Ile208Met + Thr211Asn + Thr223Gln
Pro204Gln + Leu209Ile + Arg218Asp
Ile208Leu + Ser216Glu + Ile220Pro
Pro204Gly + Gly205Gln + Thr206Glu
Ile208Ser + Leu209His + Gly214Pro
Gly203Pro + Ile208Gln + Trp212Ser
Gly205Asn + Gly215Glu + Gly222Pro

Gly203Ser + Thr217Gln + Ile220Leu
Leu209Ser + Gly214Ser + Gly222Gln
Ile208Gly + Leu209Pro + Ser221Asp
Gly205Asn + Thr206Ser + Ile220Gly
Ser207Glu + Leu209Met + Gly222Asn
Gly203Ser + Trp212Gln + Ser216Asp
Gly205Asn + Ile208Cys + Thr211Gln
Thr211Gly + Trp212Gly + Thr223Glu
Gly205Asn + Gly214Pro + Ser221Glu
Gly205Ser + Trp212Ala + Ser216Asp
Gly203Gln + Arg218Glu + Ile220Leu
Ser207Asp + Thr211Asn + Thr223Gly
Trp212Cys + Gly215Asn + Thr223Pro
Gly205Asn + Gly214Ser + Arg218Glu
Gly214Pro + Thr217Glu + Gly222Asn
Thr206Gln + Ile208Asn + Thr211Ser
Gly203Gln + Gly214Pro + Ser219Glu
Trp212His + Ile213Gln + Arg218Glu
Ile208Ala + Ile213Ala + Ser221Glu
Pro204Asn + Ile208Leu + Trp212Pro
Gly205Asn + Ser219Asp + Ile220His
Gly205Gln + Ile208Leu + Thr211Gln
Trp212Tyr + Ile213Gly + Gly215Gln
Trp212Met + Gly215Glu + Gly222Asn
Thr206Ser + Thr211Ser + Thr217Asn
Ile208Leu + Ile213Ser + Ile220Pro
Ile208Ser + Gly214Pro + Gly222Asn
Thr211Ser + Ser216Glu + Thr223Pro
Gly203Ser + Thr217Pro + Thr223Gly
Gly203Ser + Trp212Ala + Gly214Asp
Gly205Asn + Ile208Val + Ser219Asp
Gly203Ser + Pro204Gln + Ser221Asp
Gly203Pro + Trp212Gly + Gly222Pro
Ile208Gln + Thr217Gly + Arg218Glu
Thr206Gln + Ser207Glu + Thr223Gly
Ser207Asp + Leu209His + Thr211Pro
Ile208Thr + Trp212Val + Ile220Met
Leu209Ala + Ser221Asp + Thr223Gln
Gly203Asn + Thr211Gly + Gly214Glu
Gly214Pro + Gly215Glu + Ile220Pro
Gly205Ser + Gly214Asn + Ser216Glu
Thr206Gln + Ile213Thr + Ser219Glu
Pro204Gly + Thr211Gly + Ser221Asp
Gly203Pro + Gly205Asn + Gly215Asp
Gly203Pro + Thr211Asn + Thr223Asn
Pro204Gly + Leu209Pro + Gly215Glu
Gly203Pro + Ile213Glu + Ile220Gly
Pro204Gln + Ile213Met + Ile220Pro
Gly214Pro + Thr217Gln + Gly222Glu
Ile208His + Ser221Glu + Thr223Asn
Gly203Pro + Gly214Asn + Arg218Asp
Ile213Ala + Ser216Asp + Gly222Ser
Gly203Asn + Thr211Gly + Thr217Asn

Leu209Met + Thr211Gln + Ser219Asp
Thr206Ser + Leu209Ile + Thr211Gly
Ile213Met + Gly215Gln + Ser221Asp
Pro204Asn + Ser207Asp + Ile220His
Gly203Gln + Ile208Val + Ser221Asp
Thr217Gln + Ile220Val + Thr223Glu
Gly205Asn + Ile208Val + Ile213His
Thr206Asn + Thr211Gly + Thr217Asp
Ser207Glu + Ile208Cys + Gly215Pro
Pro204Asn + Gly205Pro + Trp212Ile
Pro204Gly + Ile208Val + Thr223Asp
Pro204Gly + Gly215Asn + Ile220His
Gly205Asn + Thr211Asn + Trp212Pro
Pro204Ser + Thr206Glu + Ile213Val
Gly203Pro + Ser207Glu + Gly214Asn
Pro204Gln + Gly214Glu + Gly215Glu
Gly205Ser + Thr217Asp + Arg218Asp
Leu209Met + Thr217Asp + Arg218Asp
Thr217Asp + Arg218Asp + Ile220Cys
Thr211Gln + Ser221Glu + Gly222Asp
Gly205Pro + Thr206Asp + Ser207Glu
Pro204Gly + Thr206Asp + Ser207Glu
Thr211Gln + Ile220Glu + Ser221Asp
Trp212Tyr + Arg218Asp + Ser219Glu
Ile208Val + Arg218Glu + Ser219Glu
Gly205Gln + Gly215Asp + Ser216Asp
Leu209Glu + Trp212Phe + Ser219Asp
Thr206Glu + Ile208Ala + Ser221Asp
Gly205Asn + Ser207Glu + Ser221Glu
Ser207Glu + Thr211Asn + Ile220Asp
Gly203Pro + Ser207Glu + Ile220Glu
Ser207Asp + Trp212His + Ser219Asp

TABLE 25

Loop 6 - Quadruple Mutation Variants

Gly203Gln + Thr206Gly + Leu209Ser + Ser219Glu
Ile208Val + Thr217Ser + Ser219Asp + Gly222Pro
Gly203Asn + Ile213Thr + Gly214Pro + Ser219Glu
Gly203Gln + Trp212Phe + Gly214Asn + Gly215Asp
Ser207Asp + Leu209Thr + Thr211Pro + Trp212Gly
Gly205Pro + Trp212Asn + Ile213His + Ile220Asp
Gly203Gln + Pro204Ser + Trp212Ile + Ile220Ser
Pro204Gln + Thr211Gly + Ser219Glu + Ile220Met
Gly203Gln + Trp212Gly + Gly215Glu + Ile220Ala
Thr206Gln + Thr211Pro + Gly215Asn + Ile220Thr
Leu209Val + Thr211Ser + Arg218Asp + Ile220Ser
Gly203Gln + Ile208Cys + Leu209Ser + Gly214Glu
Gly205Gln + Ser207Glu + Gly215Gln + Ile220Ala
Ile208Ala + Leu209Val + Ser216Glu + Thr223Pro
Pro204Gly + Ile208His + Ser219Glu + Ile220Ser
Thr206Asp + Ile208Gly + Thr217Asn + Thr223Ser

Gly203Asn + Leu209Ser + Gly214Ser + Ser221Asp
Ile213Asp + Gly214Ser + Ile220Gln + Gly222Asn
Trp212Thr + Ile213Gly + Gly214Ser + Ile220Ala
Gly203Asn + Leu209Asp + Trp212Phe + Gly222Gln
Thr206Asn + Thr211Ser + Ile213Gly + Ser219Asp
Pro204Asn + Gly205Gln + Thr206Asn + Ser207Glu
Gly203Gln + Gly205Asn + Ser207Asp + Leu209Thr
Gly205Gln + Ile213Val + Gly214Pro + Arg218Glu
Thr206Pro + Ile213Pro + Gly222Pro + Thr223Asp
Gly203Asn + Gly214Asn + Ser221Asp + Thr223Gly
Ile213Asn + Ser216Asp + Ile220Asn + Gly222Asn
Pro204Ser + Leu209Pro + Ile213Glu + Ile220Pro
Gly203Gln + Gly205Ser + Leu209Ala + Gly215Ser
Gly203Gln + Ser207Asp + Thr211Gly + Gly222Asn
Pro204Gln + Leu209His + Thr217Gly + Ser219Glu
Thr206Asp + Ile208Thr + Leu209Ala + Thr217Asn
Ile208Thr + Leu209Gly + Arg218Asp + Ile220Leu
Pro204Asn + Ile208Val + Leu209His + Arg218Glu
Ile208Asn + Thr211Gln + Ser219Glu + Gly222Gln
Thr206Gly + Ser207Glu + Ile208Thr + Ile213Gly
Leu209Glu + Gly214Gln + Ile220Val + Gly222Gln
Gly205Gln + Gly214Asn + Thr217Gln + Thr223Glu
Gly203Gln + Leu209Val + Gly215Pro + Ile220Glu
Thr211Gln + Trp212Gln + Gly215Asn + Ser219Glu
Pro204Ser + Ile208Ser + Gly222Glu + Thr223Gln
Pro204Gly + Ile208Ser + Gly215Gln + Ser221Asp
Gly203Gln + Thr211Gly + Ile213Leu + Gly214Asn
Pro204Asn + Thr211Ser + Gly214Asp + Thr217Asn
Pro204Gly + Trp212Met + Gly215Glu + Gly222Asn
Thr206Glu + Ile208Leu + Ile213Ser + Ile220Pro
Thr206Gly + Trp212Leu + Gly214Asp + Gly222Ser
Gly203Ser + Gly214Asp + Thr217Pro + Thr223Gly
Gly203Ser + Pro204Asn + Ile208Ala + Gly215Glu
Gly203Ser + Thr211Ser + Ser219Glu + Gly222Asn
Ser207Glu + Ile208Gly + Thr211Pro + Ile220Pro
Gly203Asn + Gly205Pro + Leu209Ala + Gly222Glu
Leu209Asn + Thr211Gly + Ile213Thr + Thr223Gln
Gly203Pro + Pro204Asn + Ile213His + Thr217Gln
Ile208Asn + Thr217Pro + Ile220Leu + Ser221Glu
Thr206Ser + Thr211Gln + Ile213Gly + Ser221Asp
Gly205Pro + Gly215Asp + Ile220His + Thr223Gly
Pro204Gln + Ile208Thr + Ser219Asp + Gly222Asn
Gly203Pro + Leu209Met + Thr211Pro + Ile220Cys
Thr206Gly + Leu209Cys + Ile213His + Ser216Asp
Ile208Gly + Leu209Met + Thr217Gly + Ile220Pro
Gly203Asn + Thr206Ser + Gly214Gln + Ser221Glu
Ile208His + Thr211Ser + Trp212Pro + Arg218Asp
Thr206Ser + Ile208Pro + Leu209Ile + Gly214Asp
Gly205Pro + Thr206Glu + Ile213His + Gly222Asn
Thr211Asn + Ile213Gln + Thr217Asp + Thr223Ser
Thr206Asn + Trp212Val + Arg218Glu + Ile220Pro
Pro204Asn + Ser207Asp + Thr211Gln + Gly214Ser
Pro204Ser + Ile213Met + Gly214Asp + Thr217Gly

Gly203Pro + Ile208Ala + Ser216Glu + Thr217Pro
Ser207Asp + Trp212Cys + Gly215Pro + Thr217Gly
Pro204Asn + Gly214Gln + Ser216Glu + Ile220His
Pro204Ser + Trp212Ala + Gly214Asp + Gly215Pro
Pro204Asn + Leu209Gly + Thr211Gly + Ile213Thr
Pro204Ser + Gly205Gln + Ile208His + Ser219Asp
Gly203Asn + Gly205Ser + Leu209Ser + Arg218Glu
Thr206Pro + Thr211Gln + Ile213Glu + Gly214Asn
Ser207Asp + Gly214Asn + Ile220Val + Gly222Asn
Trp212Tyr + Ile213Val + Gly214Asn + Gly222Ser
Gly205Pro + Ile208Gly + Ser216Asp + Ile220His
Gly203Asn + Leu209Gly + Gly214Pro + Arg218Asp
Pro204Gln + Ile208Cys + Ile213Gly + Gly215Glu
Pro204Asn + Gly205Pro + Leu209Ala + Thr211Pro
Gly203Pro + Ile213Ala + Thr217Gly + Arg218Glu
Gly203Gln + Pro204Gly + Ile220Gly + Ser221Glu
Gly205Asn + Ile213Gly + Gly214Pro + Gly222Ser
Gly203Asn + Thr211Gln + Gly214Glu + Gly222Gln
Ile213Thr + Gly215Gln + Ser219Asp + Ile220Pro
Gly203Asn + Pro204Ser + Arg218Asp + Thr223Pro
Gly203Ser + Thr211Gln + Arg218Glu + Ile220Val
Gly203Ser + Pro204Ser + Ile208Ser + Thr211Gln
Pro204Gly + Gly214Asn + Gly215Glu + Thr223Asn
Ile208Cys + Thr211Pro + Trp212Ser + Gly215Pro
Pro204Asn + Ile213Met + Thr217Glu + Ile220Gln
Pro204Gly + Gly205Pro + Ile208Gly + Thr211Pro
Gly203Ser + Thr206Gly + Ile208Asn + Ser219Asp
Pro204Asn + Ile213Glu + Gly215Pro + Thr217Asn
Gly203Asn + Thr211Gln + Arg218Glu + Ile220Gly
Gly203Gln + Ile208Cys + Gly215Ser + Arg218Asp
Pro204Gln + Gly205Gln + Gly214Ser + Thr223Gly
Pro204Gln + Leu209Met + Trp212Phe + Ser219Glu
Gly205Asn + Ile208Cys + Leu209Ile + Ile220Asn
Gly203Pro + Leu209Cys + Thr217Gly + Ile220Gly
Gly205Asn + Thr206Gly + Ile208Asn + Ser216Glu
Pro204Gln + Thr206Pro + Ser207Glu + Gly214Asn
Gly203Gln + Ile208Met + Trp212His + Thr223Pro
Pro204Asn + Leu209Val + Thr211Asn + Gly215Ser
Gly203Asn + Ile208His + Ser221Asp + Gly222Ser
Pro204Asn + Leu209Pro + Ser219Glu + Ile220Glu
Thr211Pro + Ile213Asn + Gly214Glu + Gly215Asp
Thr211Gln + Gly215Ser + Thr217Asp + Arg218Asp
Gly205Asn + Thr217Asp + Arg218Asp + Ile220Gln
Ile208Thr + Leu209Thr + Ser221Asp + Gly222Glu
Gly203Asn + Gly205Asn + Ser221Asp + Gly222Glu
Thr206Ser + Trp212Pro + Ser221Asp + Gly222Asp
Ile208Pro + Thr211Ser + Gly222Asp + Thr223Asp
Pro204Ser + Trp212Met + Gly222Glu + Thr223Asp
Gly203Gln + Thr206Glu + Ser207Glu + Thr211Gln
Gly203Asn + Thr206Glu + Ser207Asp + Thr211Asn
Thr206Asp + Ser207Asp + Ile220Thr + Thr223Pro
Gly203Gln + Thr217Ser + Ile220Glu + Ser221Asp
Gly205Ser + Thr211Gln + Arg218Glu + Ser219Asp

Leu209Gly + Gly214Ser + Arg218Glu + Ser219Glu
Ile208Gln + Gly214Pro + Ser216Asp + Thr217Glu
Ile208Gly + Ile213Met + Gly215Glu + Ser216Glu
Ile208Ser + Leu209Asp + Thr211Gly + Ser219Asp
Pro204Gln + Ile208Thr + Leu209Asp + Ser219Asp
Thr206Asp + Trp212Gln + Ser221Asp + Thr223Gly
Gly203Asn + Thr206Asp + Trp212Phe + Ser221Glu
Pro204Gln + Thr206Glu + Leu209Ile + Ser221Glu
Pro204Gln + Gly205Gln + Ser207Glu + Ser221Glu
Ser207Asp + Ile208Leu + Trp212Ile + Ser221Glu
Ser207Glu + Leu209Met + Ile220Glu + Thr223Ser
Thr217Asn + Arg218Asp + Ser219Asp + Ile220Asp
Pro204Asn + Ser207Asp + Ser219Glu + Gly222Gln
Pro204Gly + Gly205Pro + Ser207Glu + Ser219Glu
Gly205Gln + Ser207Asp + Trp212Val + Ser219Glu
Ser207Glu + Ile208Ser + Ser219Glu + Thr223Gly
Thr206Asp + Thr217Ser + Ile220Asp + Ser221Glu
Trp212Gln + Gly214Asp + Ser216Glu + Ile220Val
Gly214Asp + Gly215Gln + Ser216Glu + Thr223Asn
Gly203Gln + Leu209Asp + Thr211Asn + Arg218Glu
Pro204Ser + Leu209Glu + Arg218Asp + Ile220Cys
Ser207Asp + Ile208Gly + Ser221Glu + Gly222Asp
Pro204Asn + Ser207Glu + Ser221Asp + Gly222Asp
Pro204Ser + Gly205Ser + Ile213Asp + Ser216Asp
Thr206Asn + Ser219Glu + Ser221Glu + Thr223Asn
Thr206Pro + Trp212Pro + Ser216Glu + Arg218Glu
Gly203Ser + Gly215Asn + Ser216Asp + Arg218Glu
Thr206Gln + Trp212Asn + Ser216Asp + Arg218Glu
Leu209Val + Trp212Ile + Ser216Asp + Arg218Glu
Pro204Gly + Ile208Pro + Ser216Asp + Arg218Asp
Ile208Gly + Thr211Asn + Ser216Glu + Arg218Glu
Trp212His + Ile213Asn + Thr217Glu + Ser219Glu
Ile208Leu + Thr211Pro + Thr217Asp + Ser219Glu
Gly203Asn + Thr217Asp + Ser219Glu + Ile220Ala
Thr206Pro + Thr217Glu + Ser219Glu + Ile220Ala
Gly203Gln + Gly215Glu + Thr217Glu + Ile220Cys
Gly203Asn + Arg218Glu + Ser219Asp + Ser221Glu
Leu209Met + Ser219Asp + Ser221Glu + Gly222Asp
Thr211Ser + Ile213Glu + Ser216Asp + Arg218Asp
Thr211Asn + Ser216Asp + Arg218Asp + Ser219Asp
Ser216Glu + Arg218Asp + Ser219Glu + Thr223Ser
Gly203Pro + Thr206Glu + Leu209Asp + Ile220Asp
Ser207Glu + Leu209Gln + Gly222Asp + Thr223Ser
Gly205Gln + Ser207Glu + Ile213Gln + Gly222Asp
Gly205Asn + Ser207Glu + Ile213Leu + Gly222Asp
Gly205Gln + Ser207Glu + Gly222Asp + Thr223Glu
Ser207Asp + Trp212Gly + Ser219Glu + Gly222Glu
Trp212Thr + Gly214Glu + Ser216Asp + Arg218Asp
Ser207Asp + Arg218Asp + Ser221Asp + Gly222Asn
Ser207Asp + Trp212His + Arg218Glu + Ser221Glu
Pro204Asn + Thr206Asp + Thr211Ser + Ser219Asp
Thr206Asn + Ile213Pro + Gly214Glu + Thr217Asp
Thr211Gln + Gly214Glu + Gly215Ser + Thr217Glu

Leu209Asp + Gly214Pro + Ser221Glu + Gly222Asp
Ser207Glu + Leu209Ala + Ile213Ala + Arg218Glu
Pro204Gln + Ser207Glu + Trp212Leu + Arg218Asp
Pro204Gly + Ile213Glu + Arg218Glu + Ser219Glu
Pro204Gly + Thr206Glu + Leu209Asp + Thr223Gly

TABLE 26

Loop 6 - Quintuple Substitution Variants

Pro204Gln + Gly205Gln + Ile208Ala + Ser216Asp + Gly222Pro
Pro204Gln + Ser207Glu + Ile208Ser + Thr211Gly + Ile220Ala
Gly205Gln + Trp212Phe + Gly214Pro + Gly215Asp + Thr217Gly
Gly203Asn + Ile208Gln + Leu209Gln + Thr211Pro + Thr223Asp
Gly203Ser + Ile208Cys + Gly214Gln + Ser219Glu + Ile220Thr
Trp212Gln + Ile213Ser + Gly214Asn + Thr217Glu + Ile220Ser
Gly205Ser + Ile208Pro + Trp212Tyr + Gly214Gln + Ser221Asp
Gly205Gln + Thr206Glu + Ile208Thr + Leu209Thr + Trp212Cys
Gly203Asn + Gly205Pro + Ile208Val + Gly214Asn + Ser219Glu
Gly203Gln + Gly205Gln + Ile208Gly + Thr217Asn + Gly222Ser
Ile208Met + Thr211Gly + Ile213Cys + Gly214Pro + Ile220Leu
Thr211Pro + Ile213Ser + Thr217Asp + Ile220Pro + Gly222Gln
Pro204Asn + Gly205Ser + Thr211Gly + Ile220Thr + Gly222Asn
Gly203Asn + Gly205Asn + Leu209His + Ile220His + Thr223Gln
Pro204Gly + Ile208Asn + Thr217Gln + Gly222Glu + Thr223Gln
Gly205Gln + Ser207Asp + Ile208Met + Ile213Cys + Gly214Pro
Gly205Asn + Thr211Gln + Trp212Met + Thr217Glu + Gly222Asn
Gly205Gln + Thr206Pro + Leu209Glu + Ile213Thr + Ile220Gly
Thr211Asn + Gly214Gln + Gly215Ser + Thr217Ser + Gly222Ser
Pro204Gly + Gly205Gln + Thr211Gly + Ser221Glu + Thr223Gln
Gly203Ser + Ile208Thr + Ile220Val + Gly222Pro + Thr223Glu
Gly205Asn + Thr206Pro + Ser216Glu + Thr217Gln +

Gly222Ser
Gly203Asn + Pro204Gln + Ile208Met + Gly215Gln + Ser219Asp
Gly205Gln + Thr206Pro + Trp212Ser + Ile213Gln + Ser221Glu
Leu209Gln + Gly214Gln + Thr217Ser + Ser221Asp + Thr223Asn
Ser207Glu + Trp212Tyr + Ile213Gly + Gly214Pro + Ile220Pro
Gly203Pro + Trp212Pro + Gly214Glu + Gly215Pro + Gly222Pro
Pro204Ser + Thr206Asn + Thr211Gly + Trp212Gln + Thr223Gln
Pro204Gly + Thr206Pro + Trp212Val + Gly214Pro + Arg218Glu
Gly203Gln + Thr211Gln + Gly215Asn + Thr217Glu + Ile220Val
Gly203Gln + Gly205Pro + Leu209Val + Thr211Gln + Gly214Asp
Pro204Asn + Ile213Thr + Gly215Gln + Ser219Asp + Ile220Pro
Pro204Gln + Thr206Asn + Ile208Met + Gly215Glu + Ile220Ala
Gly203Ser + Gly205Pro + Ile208Asn + Leu209Glu + Thr211Gln
Pro204Gly + Gly205Gln + Thr211Ser + Trp212Met + Thr217Pro
Gly205Ser + Thr206Gly + Ile208Val + Gly214Asp + Thr223Pro
Ser207Glu + Ile208Ala + Gly215Pro + Thr217Gln + Thr223Pro
Gly203Ser + Gly205Gln + Trp212Asn + Ile213Ser + Thr223Pro
Pro204Gln + Ile208Ala + Leu209Gln + Thr211Ser + Gly214Ser
Pro204Gln + Ile208His + Thr211Gln + Gly215Pro + Ser216Asp
Pro204Gln + Leu209Ile + Thr211Ser + Gly214Asp + Ile220Cys
Leu209Ile + Thr211Gln + Trp212Asn + Ile213Val + Thr217Asp
Pro204Asn + Ile208His + Leu209Val + Thr211Asn + Gly222Ser
Gly203Gln + Pro204Asn + Gly205Asn + Ile213Ala + Gly214Asp
Ser207Asp + Thr211Gln + Trp212Gly + Gly222Gln + Thr223Gly
Gly205Gln + Ile208Gly + Thr211Gln + Ile220Ser + Gly222Asp
Gly205Pro + Ile208Pro + Leu209Val + Ile220Leu + Gly222Glu
Pro204Gly + Thr206Glu + Leu209Cys + Ile213His + Ile220Met

Leu209Gly + Trp212Val + Ser216Asp + Ile220Cys + Thr223Ser
Gly205Gln + Leu209Val + Thr211Asn + Gly214Pro + Gly222Asp
Ile208Ala + Leu209His + Gly215Asn + Ile220Val + Gly222Glu
Ser207Glu + Ile208Pro + Thr211Asn + Ile213His + Gly222Ser
Ile208His + Leu209Cys + Thr217Pro + Ile220Glu + Gly222Gln
Pro204Gly + Gly205Ser + Ile208Ala + Trp212Leu + Thr223Asn
Gly203Pro + Gly205Pro + Trp212Ala + Gly215Ser + Arg218Glu
Pro204Ser + Trp212Ala + Ile213Thr + Gly215Asn + Ser216Glu
Thr211Asn + Trp212Thr + Ser216Glu + Gly222Gln + Thr223Pro
Thr206Gln + Ile208Pro + Thr211Gly + Ile213Leu + Gly215Asp
Gly203Asn + Ile220Met + Ser221Glu + Gly222Gln + Thr223Asn
Pro204Gln + Thr206Gly + Trp212Gly + Gly215Asp + Ile220Cys
Gly203Asn + Pro204Gln + Trp212Pro + Ile220Gly + Thr223Asp
Pro204Ser + Thr206Ser + Ile208Leu + Thr211Ser + Gly222Pro
Gly203Ser + Ile208Thr + Leu209His + Thr211Gln + Ile220Glu
Pro204Asn + Thr211Pro + Gly214Gln + Gly215Glu + Thr217Ser
Pro204Asn + Thr211Pro + Thr217Asp + Ile220Val + Thr223Gln
Gly203Ser + Ile208Gln + Leu209Asn + Thr211Ser + Trp212Asn
Pro204Asn + Ile208His + Thr211Gln + Ile220Gly + Gly222Asp
Gly205Pro + Trp212Val + Gly215Pro + Ser216Asp + Thr217Pro
Gly203Gln + Ile208Ala + Thr211Pro + Gly214Ser + Arg218Glu
Gly203Asn + Pro204Gln + Leu209Met + Trp212Ser + Thr217Glu
Gly205Pro + Ser207Glu + Ile208Met + Leu209Met + Ile220Asn
Thr211Gly + Trp212Asn + Ser219Glu + Ile220Asp + Thr223Pro
Ile208Met + Ile213Thr + Thr217Asp + Arg218Glu + Thr223Gly
Gly203Ser + Ile208Gln + Thr217Glu + Arg218Asp + Gly222Pro
Gly205Gln + Thr217Glu + Arg218Glu + Ile220His +

Thr223Pro
Gly203Ser + Ile213Gln + Thr217Asp + Arg218Asp + Gly222Gln
Gly203Pro + Pro204Gly + Ile208Gly + Ser221Asp + Gly222Asp
Gly203Asn + Thr211Gln + Ile213Asp + Gly214Glu + Gly222Gln
Thr206Glu + Ser207Glu + Leu209Asn + Thr211Gln + Thr217Asn
Gly203Gln + Leu209Met + Thr211Pro + Ile220Glu + Ser221Glu
Ile208Val + Leu209His + Thr211Gln + Arg218Glu + Ser219Glu
Gly203Pro + Pro204Asn + Leu209His + Arg218Asp + Ser219Asp
Ile208Ala + Trp212Gln + Arg218Glu + Ser219Glu + Ile220Thr
Leu209Gly + Thr211Pro + Gly215Asn + Ser216Glu + Thr217Asp
Ile208Asn + Leu209Thr + Ile213His + Ser216Glu + Thr217Glu
Leu209Asn + Gly215Glu + Ser216Asp + Ile220Asn + Thr223Asn
Gly203Ser + Pro204Gly + Gly215Glu + Ser216Asp + Thr223Ser
Pro204Gln + Ile208Cys + Ile213Cys + Gly215Glu + Ser216Glu
Pro204Gln + Gly205Asn + Thr206Asp + Ser207Glu + Ser221Glu
Gly203Gln + Pro204Asn + Thr206Asp + Ile208Cys + Ser221Glu
Pro204Gly + Thr206Glu + Ile208Gln + Trp212His + Ser221Glu
Thr206Asp + Ile208Met + Trp212Met + Ile213Met + Ser221Glu
Ser207Asp + Thr211Gln + Trp212Ala + Ile213Pro + Ser221Asp
Thr206Gln + Ser207Asp + Leu209Val + Thr211Gly + Ser221Asp
Pro204Ser + Ser207Glu + Ile208Thr + Trp212Ile + Ser221Asp
Ser207Glu + Leu209Ala + Thr217Gly + Ile220Ala + Ser221Asp
Ser207Asp + Thr211Pro + Trp212Cys + Gly215Pro + Ser221Glu
Gly205Pro + Ser207Glu + Trp212Asn + Ser221Glu + Thr223Ser
Gly205Gln + Ser207Glu + Thr211Gly + Thr217Gly + Ser221Glu
Thr206Gln + Ser207Glu + Ile208Ala + Leu209Cys + Ile220Glu
Thr211Gln + Arg218Glu + Ser219Asp + Ile220Asp + Gly222Ser

Gly205Asn + Thr206Pro + Ser207Asp + Ser219Glu + Gly222Gln
Pro204Ser + Ser207Asp + Ser219Asp + Ile220Met + Thr223Pro
Thr206Asn + Ser207Glu + Ile208Thr + Ile213Gly + Ser219Glu
Gly205Gln + Leu209Asn + Thr217Asp + Arg218Glu + Ser219Asp
Pro204Gly + Ile208Val + Ile213Asp + Gly214Glu + Ser216Asp
Pro204Ser + Ile220His + Ser221Glu + Gly222Glu + Thr223Glu
Thr206Asp + Trp212Val + Ile220Glu + Ser221Asp + Thr223Pro
Thr206Glu + Ser207Asp + Gly214Asn + Ile220Asp + Thr223Asn
Ile208Leu + Leu209Val + Thr211Ser + Gly214Asp + Ser216Glu
Thr211Ser + Ile213His + Gly214Asp + Ser216Asp + Ile220Gln
Gly205Pro + Thr206Ser + Leu209Glu + Thr217Asn + Arg218Glu
Gly205Ser + Leu209Asp + Gly214Ser + Gly215Ser + Arg218Asp
Ser207Glu + Leu209Gly + Trp212Phe + Ser219Glu + Ser221Glu
Gly203Pro + Pro204Asn + Thr211Ser + Ile213Glu + Ser216Glu
Thr206Glu + Ser207Asp + Ile208Ala + Gly215Pro + Ser219Glu
Thr206Glu + Leu209Ser + Trp212Ile + Ile220Thr + Gly222Glu
Thr206Asp + Ser207Asp + Ile208Leu + Ile213Pro + Gly222Glu
Gly203Ser + Thr206Asp + Ser207Glu + Ile220Gln + Gly222Glu
Thr211Gly + Ile213Ala + Gly214Glu + Ser216Glu + Thr217Asp
Ser207Asp + Thr211Gln + Arg218Glu + Ser219Glu + Ile220Val
Ser207Glu + Leu209Glu + Gly214Gln + Ile220Val + Gly222Gln
Ser207Glu + Leu209Glu + Thr211Gln + Gly214Gln + Thr217Gln
Gly205Gln + Ser207Glu + Thr211Ser + Arg218Asp + Ile220Glu
Leu209Glu + Thr217Pro + Ser219Asp + Ser221Glu + Gly222Ser
Thr211Gly + Gly214Asn + Ser219Glu + Ile220Leu + Ser221Asp
Gly203Pro + Ile208Gln + Gly214Pro + Ser219Asp + Ser221Glu
Pro204Asn + Leu209Ile + Ser219Glu + Ser221Asp +

Gly222Ser
Thr211Ser + Trp212Gln + Gly214Gln + Ser219Asp + Ser221Asp
Leu209Ala + Gly214Pro + Ser219Glu + Ile220Met + Ser221Glu
Pro204Gly + Gly205Gln + Ile213Val + Ser216Asp + Arg218Glu
Pro204Gly + Thr206Pro + Thr211Asn + Ser216Glu + Arg218Glu
Thr206Asp + Ile208Thr + Leu209Ala + Thr217Asn + Ile220Asp
Pro204Gln + Thr206Glu + Ile208Pro + Trp212His + Ile220Glu
Thr206Gln + Thr211Ser + Thr217Asp + Ser219Asp + Thr223Pro
Gly203Ser + Pro204Ser + Gly205Pro + Gly215Asp + Thr217Glu
Gly203Pro + Pro204Gln + Gly215Glu + Thr217Glu + Gly222Gln
Pro204Ser + Leu209Met + Gly215Glu + Thr217Asp + Thr223Asn
Pro204Gly + Trp212Leu + Gly214Ser + Ser221Asp + Thr223Asp
Leu209Ser + Thr211Asn + Arg218Asp + Ile220Glu + Ser221Glu
Ile208Gly + Leu209Asn + Ser219Asp + Ser221Glu + Gly222Asp
Pro204Gly + Ile208Leu + Gly215Asp + Ser216Glu + Arg218Asp
Pro204Gln + Ile208Gly + Gly215Asp + Ser216Glu + Arg218Glu
Thr206Gly + Ile213Ser + Ser216Glu + Arg218Asp + Ser219Glu
Pro204Gln + Thr206Glu + Ser207Asp + Thr211Asn + Thr223Glu
Pro204Gly + Gly205Ser + Ser207Glu + Arg218Asp + Ser221Asp
Thr206Asp + Ile213Thr + Gly214Ser + Ser219Asp + Gly222Glu
Gly203Asn + Thr206Asn + Ile213Pro + Gly214Glu + Thr217Asp
Pro204Ser + Ile213Gly + Gly214Glu + Thr217Asp + Ile220Ala
Ser207Glu + Thr217Asp + Arg218Asp + Ile220Ala + Thr223Ser
Ser207Asp + Leu209Ser + Trp212Val + Gly215Ser + Arg218Glu
Pro204Asn + Ser207Glu + Leu209Val + Trp212Phe + Arg218Asp
Gly205Ser + Ser207Glu + Gly215Gln + Arg218Glu + Thr223Asn
Gly205Ser + Leu209Asp + Ile213Pro + Gly215Asn + Ser221Asp

Gly205Pro + Ser207Glu + Leu209Glu + Thr217Ser + Gly222Asp
Ser207Asp + Leu209Asp + Thr217Gly + Gly222Asp + Thr223Ser
Gly203Asn + Ile208Ser + Leu209Asp + Ser216Glu + Ser219Asp
Leu209Ala + Trp212Ser + Gly214Glu + Gly215Glu + Arg218Glu
Gly205Gln + Thr206Glu + Ile213Gln + Arg218Glu + Ser221Glu
Pro204Gly + Leu209Asn + Gly215Pro + Thr217Asp + Ile220Asp
Thr206Pro + Trp212Ala + Arg218Asp + Ser219Glu + Gly222Asp
Gly203Asn + Gly205Pro + Trp212Asn + Arg218Glu + Ser221Asp
Thr206Gly + Ile208Ala + Trp212Tyr + Arg218Asp + Ser221Glu
Gly205Gln + Thr211Ser + Arg218Asp + Ser221Glu + Thr223Pro
Gly205Pro + Thr211Pro + Trp212Pro + Arg218Glu + Ser221Glu
Gly203Ser + Arg218Asp + Ile220Asn + Ser221Asp + Thr223Ser
Trp212His + Ile213Asn + Gly214Glu + Thr217Glu + Ser219Glu
Gly205Pro + Ile213Pro + Gly215Asp + Arg218Glu + Ser219Asp
Pro204Ser + Gly215Asp + Arg218Glu + Ser219Glu + Gly222Asn
Ser207Glu + Gly214Gln + Ser216Asp + Arg218Glu + Gly222Gln
Ser207Asp + Gly215Pro + Ser216Glu + Thr217Asn + Arg218Asp
Gly205Ser + Ile208Cys + Leu209Ala + Gly215Asp + Arg218Asp
Gly203Asn + Ser207Asp + Ser216Glu + Ser219Asp + Gly222Pro
Ser207Glu + Leu209Ile + Ser216Glu + Thr217Gly + Ser219Glu
Leu209Asp + Trp212Pro + Gly214Glu + Ser216Glu + Ile220Cys
Gly203Gln + Thr206Gly + Leu209Ser + Ser216Glu + Ser219Glu
Ile208Leu + Gly214Asn + Ser216Glu + Thr217Ser + Ser219Glu
Leu209Ser + Ser216Glu + Thr217Ser + Ser219Glu + Ile220His
Pro204Gln + Leu209Pro + Gly214Ser + Ser216Glu + Ser219Asp
Thr206Gly + Ile208Met + Gly215Asp + Arg218Asp + Ile220Glu

TABLE 27

Loop 6 - Sextuple Substitution Variants

Gly203Asn + Thr206Asn + Ile208Asn + Trp212Asn + Ile213Leu + Ser221Glu
Pro204Gln + Gly205Gln + Ile208Ala + Leu209Pro + Ser216Asp + Gly222Pro
Gly203Gln + Gly205Asn + Gly215Ser + Thr217Ser + Ile220Met + Ser221Asp
Ile208Pro + Leu209Ala + Gly215Gln + Arg218Asp + Ile220Val + Thr223Ser
Gly203Ser + Leu209Cys + Trp212Tyr + Ser216Asp + Thr217Asn + Ile220Val
Gly203Gln + Gly205Ser + Leu209Ala + Ile213Met + Gly215Asn + Gly222Asn
Gly203Pro + Gly205Ser + Leu209Ala + Trp212Ala + Gly214Gln + Ser219Asp
Gly203Pro + Leu209Ile + Thr211Pro + Ile213Val + Gly215Pro + Ile220Pro
Thr206Ser + Ile208Asn + Thr211Gln + Ile213Gly + Ile220Leu + Ser221Asp
Pro204Ser + Gly205Pro + Ile208Thr + Gly215Asp + Ile220His + Thr223Gly
Gly203Pro + Pro204Gln + Leu209Met + Thr211Pro + Ser219Asp + Ile220Cys
Pro204Asn + Thr206Asn + Ser207Asp + Thr211Gln + Gly214Ser + Ile220Pro
Gly203Ser + Leu209Pro + Trp212Met + Ile213Cys + Thr217Pro + Arg218Glu
Pro204Asn + Gly205Ser + Ile208Ala + Ile213Pro + Gly215Ser + Ile220Gly
Gly203Ser + Gly205Asn + Ser207Glu + Thr211Ser + Trp212Val + Thr217Asn
Gly203Gln + Pro204Asn + Ile208His + Leu209Pro + Ile213Leu + Gly222Asn
Gly205Asn + Thr206Gly + Ile208Pro + Thr211Pro + Trp212Ile + Thr223Asn
Gly205Gln + Thr206Ser + Ile208Gln + Ser221Asp + Gly222Gln + Thr223Pro
Ile208Leu + Trp212Asn + Ile213Thr + Gly214Ser + Thr217Gln + Ser219Glu
Gly203Gln + Pro204Gln + Leu209His + Ser219Glu + Gly222Asn + Thr223Gln
Pro204Gln + Gly205Asn + Ile208Cys + Leu209Ile + Ile220Asn + Thr223Glu
Gly203Pro + Gly205Gln + Thr206Gly + Thr211Gln + Trp212Pro + Gly215Glu
Thr206Gln + Ile208Leu + Thr211Gln + Gly214Asn + Ile220Asn + Thr223Asn
Ile208Val + Leu209Cys + Thr211Ser + Ile213Gly + Gly214Asp + Thr223Gly

Pro204Ser + Gly205Asn + Leu209Ser + Thr217Gly + Arg218Glu + Gly222Ser
Pro204Ser + Gly205Gln + Leu209His + Thr211Gln + Gly214Ser + Ser221Glu
Ile208His + Leu209Cys + Thr211Ser + Trp212Ser + Gly214Glu + Gly222Asn
Ile208Gln + Leu209Gly + Trp212Phe + Thr217Pro + Ile220Thr + Gly222Glu
Gly205Pro + Ser207Glu + Ile208Met + Leu209Met + Trp212Cys + Ile220Asn
Pro204Ser + Thr206Asn + Ile208Gln + Trp212Phe + Ser219Glu + Thr223Gly
Gly205Pro + Ile208Cys + Trp212Ile + Thr217Gln + Ile220Leu + Gly222Pro
Pro204Ser + Gly205Ser + Ile208Cys + Ile213Pro + Ser221Asp + Thr223Gln
Pro204Ser + Thr206Gln + Trp212Met + Gly214Ser + Ser221Asp + Thr223Ser
Gly205Ser + Ile208Gln + Ile213Cys + Gly215Pro + Thr217Gln + Thr223Asp
Pro204Asn + Gly205Pro + Ile208Ser + Ile213Asp + Ile220Thr + Gly222Gln
Pro204Asn + Ile208Ala + Leu209Ser + Thr211Gly + Gly215Ser + Ser219Glu
Gly203Gln + Gly205Gln + Thr206Gln + Ser207Glu + Ile208Ala + Ile220Ala
Leu209Ser + Thr211Gly + Trp212Tyr + Ile213His + Ser216Asp + Thr223Pro
Pro204Gln + Thr206Ser + Ile208Met + Leu209Cys + Thr211Ser + Gly214Asp
Gly203Asn + Leu209Gln + Thr211Ser + Gly215Pro + Ile220Cys + Gly222Pro
Leu209His + Thr211Asn + Trp212Ile + Ile213Ala + Ser219Glu + Thr223Gly
Thr206Gln + Ile208His + Trp212Gln + Gly215Ser + Ser221Asp + Thr223Gln
Ile208Cys + Leu209Met + Trp212Ser + Ile213Gln + Ser216Glu + Thr223Pro
Gly205Ser + Leu209Met + Trp212Gly + Arg218Glu + Ile220Gly + Gly222Asn
Pro204Asn + Trp212Gln + Gly215Ser + Ser216Glu + Thr217Asn + Ile220Asn
Gly203Pro + Ile208Val + Leu209Asp + Trp212Ala + Ile220Gln + Thr223Gly
Gly203Gln + Gly205Pro + Ile208Gly + Trp212Ser + Thr217Asp + Gly222Asn
Gly205Ser + Leu209Thr + Thr217Gln + Ile220Met + Gly222Gln + Thr223Glu
Gly203Asn + Pro204Ser + Gly205Pro + Trp212Pro + Gly215Gln + Thr217Glu
Pro204Ser + Gly205Ser + Leu209Asn + Ser216Glu + Ile220Leu + Gly222Asn
Gly203Gln + Leu209Thr + Trp212Leu + Ile213Gly +

Ser219Glu + Ile220His
Ile208Gln + Trp212Gly + Ile213Asn + Thr217Pro + Gly222Ser + Thr223Asp
Pro204Ser + Ile208His + Thr211Gly + Ile213Met + Gly214Gln + Ser216Asp
Gly203Asn + Gly205Pro + Leu209Glu + Trp212Ile + Ile213Val + Gly215Pro
Gly203Asn + Pro204Gln + Leu209Met + Trp212Ser + Thr217Glu + Arg218Glu
Leu209Gln + Trp212Gln + Gly214Asn + Gly215Ser + Gly222Glu + Thr223Glu
Gly205Pro + Thr211Gly + Trp212Val + Arg218Glu + Ser219Asp + Ile220Pro
Gly203Asn + Pro204Gln + Gly205Gln + Gly214Pro + Arg218Glu + Ser219Asp
Gly205Pro + Thr211Gln + Gly215Asn + Thr217Ser + Arg218Glu + Ser219Glu
Pro204Asn + Gly205Ser + Ile208Gly + Thr217Ser + Arg218Glu + Ser219Asp
Thr206Pro + Gly214Ser + Thr217Gln + Arg218Glu + Ser219Glu + Thr223Asn
Thr206Gln + Leu209Glu + Thr211Asn + Trp212Ile + Gly215Ser + Ser219Asp
Leu209Glu + Thr211Gln + Ile213Val + Gly215Gln + Ser219Glu + Gly222Pro
Pro204Gln + Ser207Glu + Ile208Pro + Thr211Gly + Ile220Pro + Ser221Asp
Pro204Asn + Thr206Gln + Ser207Glu + Ile208Ala + Leu209Cys + Ile220Glu
Thr206Glu + Thr211Gln + Ile213Pro + Gly214Asn + Ser221Asp + Gly222Asp
Gly203Asn + Pro204Gln + Leu209Asp + Gly215Pro + Ser219Asp + Ile220Asp
Ser207Glu + Ile208Ala + Thr211Gly + Trp212Ser + Ser219Glu + Ile220Gln
Ser207Glu + Leu209Gly + Thr211Pro + Gly215Asn + Ser219Glu + Ile220Val
Ser207Glu + Ile208Asn + Leu209Cys + Gly215Asn + Ser219Glu + Thr223Ser
Gly203Asn + Gly205Ser + Ser207Asp + Thr217Ser + Ser219Asp + Ile220Leu
Gly203Asn + Pro204Asn + Ser207Asp + Leu209Thr + Ser219Asp + Thr223Gln
Gly203Pro + Ile208Pro + Thr211Ser + Ser221Asp + Gly222Asp + Thr223Asp
Gly205Ser + Ser207Glu + Leu209Glu + Thr217Ser + Ser219Glu + Gly222Asn
Thr206Pro + Leu209Ser + Thr211Pro + Ile213Glu + Gly215Glu + Ile220Ala
Gly203Ser + Ile208Ser + Leu209His + Trp212Gly + Gly214Asp + Ser216Glu
Gly203Ser + Gly205Ser + Trp212Ala + Gly214Glu + Gly215Ser + Ser216Asp

Gly205Gln + Ser207Glu + Ile208Ser + Leu209Gly + Ser219Asp + Ser221Glu
Thr206Pro + Ser207Glu + Ile208His + Trp212Ala + Ser219Glu + Ser221Asp
Gly205Ser + Ser207Asp + Leu209Cys + Thr217Pro + Ser221Asp + Gly222Glu
Ser207Glu + Leu209Asp + Thr211Gln + Trp212Gln + Thr217Gln + Ile220Glu
Leu209Asp + Thr211Gln + Gly214Pro + Thr217Glu + Ser219Glu + Thr223Gln
Gly203Ser + Thr206Glu + Ser207Glu + Gly215Ser + Ser219Asp + Ile220Thr
Gly205Ser + Thr206Glu + Ser207Glu + Thr211Gly + Ile220Val + Gly222Glu
Thr206Glu + Ser207Asp + Leu209Ser + Trp212Ile + Ile220Thr + Gly222Glu
Gly203Pro + Ser207Glu + Leu209Asn + Thr217Gln + Arg218Asp + Ser219Asp
Gly205Gln + Thr206Gln + Ser207Asp + Arg218Glu + Ser219Asp + Ile220Pro
Pro204Ser + Gly205Gln + Ser207Asp + Thr217Asn + Arg218Asp + Ser219Asp
Gly203Pro + Thr206Pro + Ser207Glu + Ile208Asn + Leu209Asp + Thr217Ser
Gly203Asn + Pro204Gln + Thr206Asp + Trp212Phe + Ser221Glu + Thr223Asp
Pro204Gln + Gly205Gln + Ser207Glu + Leu209Glu + Trp212Met + Ser221Glu
Gly203Asn + Trp212Phe + Gly214Ser + Ser219Asp + Ser221Glu + Thr223Gln
Gly205Gln + Thr206Gln + Ile208Pro + Thr217Pro + Ser219Asp + Ser221Asp
Gly203Asn + Thr211Asn + Gly215Pro + Ser219Asp + Ser221Asp + Gly222Asn
Pro204Ser + Trp212His + Gly214Gln + Ser219Glu + Ser221Glu + Gly222Gln
Gly203Pro + Thr211Gln + Ile213Met + Ser219Glu + Ser221Asp + Gly222Ser
Ile208Leu + Thr211Gly + Gly214Pro + Gly215Asn + Ser216Glu + Arg218Asp
Ile208Ala + Leu209Pro + Trp212Pro + Ser216Glu + Arg218Asp + Thr223Pro
Gly205Gln + Ile208Asn + Gly215Ser + Ser216Glu + Arg218Glu + Thr223Asn
Gly203Ser + Thr206Gly + Gly214Pro + Gly215Gln + Ser216Asp + Arg218Asp
Gly203Ser + Thr206Pro + Trp212Pro + Gly215Asn + Ser216Glu + Arg218Glu
Gly203Asn + Gly205Asn + Leu209Val + Ile220Glu + Gly222Glu + Thr223Ser
Pro204Gly + Gly205Pro + Leu209Ile + Ile213Met + Ser221Asp + Thr223Glu
Gly205Ser + Trp212Tyr + Gly214Pro + Arg218Glu +

Ser219Asp + Ser221Asp
Gly205Gln + Ile208Ala + Leu209Cys + Arg218Glu + Ser219Asp + Ser221Asp
Gly205Gln + Leu209Val + Trp212Cys + Ile220Asp + Ser221Glu + Thr223Asp
Pro204Gln + Thr211Gln + Gly215Glu + Thr217Asp + Arg218Glu + Thr223Asn
Gly203Gln + Leu209Asn + Ser216Asp + Arg218Glu + Ser219Asp + Thr223Pro
Ile208Ala + Leu209Pro + Gly214Ser + Ser216Asp + Arg218Glu + Ser219Asp
Pro204Asn + Ile208Gly + Thr211Gln + Ser216Asp + Arg218Glu + Ser219Glu
Gly203Ser + Ile208Ala + Trp212Ser + Ser216Glu + Arg218Asp + Ser219Glu
Gly205Pro + Thr211Ser + Ser216Glu + Thr217Asp + Ser219Asp + Gly222Asn
Pro204Gln + Leu209Ala + Gly215Gln + Ser216Glu + Thr217Asp + Ser219Asp
Pro204Asn + Ser207Glu + Thr211Gln + Gly214Ser + Gly222Glu + Thr223Asn
Gly205Asn + Ser207Asp + Trp212His + Gly214Ser + Gly222Glu + Thr223Glu
Gly203Ser + Ser207Glu + Thr211Ser + Ile213Ala + Arg218Asp + Ser221Asp
Gly203Gln + Thr206Glu + Trp212Ile + Ile213Asn + Ser219Asp + Gly222Glu
Ile208Ala + Leu209Met + Thr211Pro + Ile213Thr + Gly214Glu + Thr217Glu
Gly203Ser + Pro204Ser + Gly205Ser + Gly214Asp + Gly215Pro + Thr217Glu
Gly203Asn + Ile208Met + Ile213Cys + Gly214Asp + Thr217Glu + Thr223Asn
Pro204Asn + Leu209Ile + Trp212Val + Ile213Asp + Gly215Asp + Arg218Glu
Ser207Asp + Ile208Met + Thr211Pro + Trp212Ala + Gly215Asn + Arg218Glu
Pro204Asn + Thr206Gln + Ser207Glu + Gly214Asn + Gly215Gln + Arg218Glu
Gly203Gln + Gly205Pro + Ser207Asp + Ile208Gly + Arg218Glu + Gly222Gln
Gly205Pro + Thr206Asn + Ser207Asp + Trp212Tyr + Arg218Asp + Ile220Thr
Pro204Asn + Gly205Pro + Ser207Glu + Ile208Gln + Thr217Pro + Arg218Asp
Thr206Gln + Ser207Glu + Trp212Ser + Thr217Asn + Arg218Glu + Ile220Ala
Gly205Pro + Thr206Glu + Leu209Thr + Gly214Gln + Arg218Asp + Ser219Glu
Gly203Ser + Pro204Asn + Ser207Asp + Gly215Gln + Thr217Asp + Ile220Asp
Ile208Gly + Thr211Gln + Ile213Glu + Arg218Glu + Gly222Ser + Thr223Gln

Gly203Pro + Thr206Gly + Gly214Gln + Ser219Glu + Ser221Asp + Thr223Asp
Thr211Gly + Trp212Phe + Ser219Asp + Ile220Pro + Ser221Glu + Thr223Glu
Leu209Ala + Ile213Ala + Ser219Asp + Ile220Gln + Ser221Asp + Thr223Asp
Pro204Gln + Leu209Glu + Trp212Met + Gly214Ser + Gly215Asp + Thr217Glu
Ile208Met + Ile213Ser + Thr217Glu + Ile220Asp + Ser221Asp + Thr223Gln
Leu209Asp + Thr211Asn + Ile213Asp + Arg218Glu + Ile220Leu + Gly222Ser
Gly203Pro + Gly205Gln + Thr217Asp + Arg218Glu + Ile220Ala + Ser221Asp
Trp212Cys + Ile213Gly + Arg218Glu + Ser219Glu + Ile220Pro + Gly222Glu
Thr206Gln + Ile208Gly + Leu209Ile + Arg218Glu + Ser219Glu + Gly222Asp
Gly203Gln + Ile208Met + Ser219Glu + Ile220Glu + Gly222Pro + Thr223Glu
Gly203Gln + Gly205Asn + Thr211Gln + Ser219Asp + Ile220Glu + Thr223Glu
Pro204Asn + Gly205Pro + Leu209Ser + Gly214Ser + Arg218Glu + Ser221Glu
Ile208Thr + Leu209Asn + Trp212Asn + Arg218Asp + Ser221Glu + Gly222Asn
Gly203Ser + Pro204Gln + Gly205Asn + Thr211Gly + Arg218Asp + Ser221Glu
Pro204Asn + Thr206Pro + Gly214Pro + Arg218Glu + Ser221Glu + Thr223Asn
Thr206Asp + Ile213Gln + Gly215Asn + Thr217Asp + Ser219Asp + Thr223Ser
Ile213Met + Gly214Asp + Gly215Ser + Thr217Glu + Ser219Asp + Ile220Ser
Pro204Asn + Gly205Gln + Ser207Asp + Gly215Pro + Ser219Glu + Thr223Glu
Pro204Ser + Thr206Gln + Ser207Glu + Thr217Gly + Ser219Asp + Thr223Asp
Pro204Gln + Trp212Leu + Gly215Glu + Thr217Pro + Arg218Asp + Ser219Asp
Gly203Asn + Thr211Gly + Gly214Asn + Gly215Pro + Ser219Asp + Gly222Asp
Gly203Pro + Trp212Ile + Gly214Asn + Ser219Glu + Ile220Pro + Gly222Glu
Thr206Glu + Leu209Ile + Gly215Asn + Ser219Asp + Ile220Cys + Thr223Asp
Gly203Asn + Ser207Asp + Gly214Gln + Ser216Glu + Arg218Asp + Ile220Thr
Ser207Glu + Ile208Cys + Ile213His + Gly214Ser + Ser216Asp + Arg218Glu
Thr206Gln + Ile208Ser + Leu209Asp + Gly215Glu + Arg218Asp + Ile220Ala
Gly205Ser + Ile213Cys + Gly214Asp + Arg218Glu +

Ser219Asp + Gly222Gln
Pro204Asn + Gly214Asp + Arg218Asp + Ser219Asp + Gly222Pro + Thr223Gln
Pro204Gly + Thr211Asn + Ile213Ala + Gly215Asp + Arg218Glu + Ile220Gly
Ser207Glu + Leu209Ile + Ser216Glu + Thr217Gly + Ser219Asp + Ile220Gly
Gly205Ser + Ser207Asp + Thr211Gly + Ser216Glu + Ser219Asp + Ile220Asn
Pro204Gln + Gly205Ser + Thr206Asn + Leu209Glu + Trp212Gly + Ser216Asp
Gly205Pro + Trp212Gly + Ile213His + Gly214Asp + Ser216Glu + Ser219Glu
Pro204Ser + Thr206Asn + Ile213Ala + Ser216Asp + Ser219Glu + Thr223Gln
Ile208Asn + Trp212Asn + Gly215Asn + Ser216Glu + Ser219Glu + Ile220Ser
Ile208Leu + Thr211Gly + Gly214Gln + Ser216Glu + Ser219Glu + Gly222Pro
Pro204Ser + Thr206Ser + Ser216Asp + Thr217Pro + Ser219Glu + Thr223Asn
Leu209Val + Trp212Cys + Gly214Ser + Arg218Glu + Ile220Glu + Thr223Asp
Gly205Gln + Trp212Met + Gly215Asp + Arg218Glu + Ile220Glu + Gly222Ser
Gly205Pro + Thr206Ser + Ser207Glu + Leu209Gly + Gly214Ser + Thr223Glu
Thr206Asp + Leu209Glu + Thr211Gln + Thr217Gln + Ile220Met + Thr223Glu
Pro204Gly + Ile208Cys + Thr217Ser + Arg218Glu + Ser221Glu + Thr223Asp
Ser207Glu + Leu209Val + Ile213Gly + Ser216Glu + Thr217Asp + Gly222Gln
Ile208Thr + Ser216Glu + Ser219Glu + Ile220Leu + Ser221Glu + Thr223Pro
Gly203Gln + Gly205Gln + Ile213Ala + Ser216Glu + Ser219Asp + Ser221Asp
Gly203Gln + Pro204Gln + Thr206Asp + Leu209Asn + Arg218Glu + Gly222Glu
Gly205Gln + Ile213Ala + Gly215Glu + Ser216Glu + Ile220Glu + Gly222Asn
Thr206Asn + Ile208Ala + Leu209Asp + Ile213Glu + Gly214Glu + Gly222Ser
Gly203Ser + Pro204Ser + Ile208Cys + Ile213Glu + Gly215Glu + Ser219Glu
Thr206Pro + Leu209Cys + Ile213Glu + Gly215Glu + Thr217Ser + Ser219Glu

TABLE 28

Loop 6 - Heptuple Substitution Mutation Variants

Pro204Asn + Leu209Thr + Thr211Gln + Trp212His +

Gly215Asp + Ile220Val + Thr223Ser
Pro204Gly + Gly205Asn + Ile208Leu + Thr211Asn + Trp212Gly + Gly214Pro + Arg218Asp
Gly203Pro + Thr206Gly + Ile208Pro + Thr211Gln + Thr217Glu + Ile220Met + Thr223Asn
Pro204Gln + Ile208Ala + Leu209Gln + Thr211Ser + Gly214Ser + Ser219Glu + Thr223Pro
Pro204Gly + Gly205Pro + Thr206Glu + Ile208Pro + Leu209Val + Ile213His + Ile220Leu
Gly205Gln + Thr206Ser + Ile208Leu + Thr211Ser + Gly214Asn + Gly222Pro + Thr223Glu
Pro204Gly + Gly205Ser + Ile208Ala + Trp212Leu + Thr217Gln + Arg218Asp + Thr223Asn
Ile208Asn + Thr211Asn + Trp212Val + Ile213Asp + Ile220Ala + Gly222Pro + Thr223Pro
Pro204Gly + Ile208Cys + Trp212Cys + Ile213Gly + Thr217Gln + Ser219Asp + Ile220Ala
Gly205Pro + Ile208Asn + Thr211Pro + Trp212Phe + Ile220Met + Ser221Glu + Gly222Ser
Gly203Gln + Pro204Ser + Thr206Ser + Ile208Val + Trp212Tyr + Ile220Gly + Ser221Glu
Pro204Ser + Gly205Pro + Leu209Met + Trp212Leu + Ile213Asp + Gly215Gln + Gly222Ser
Gly205Pro + Ile208Pro + Leu209Thr + Thr211Ser + Trp212Asn + Ser216Glu + Ile220Ala
Gly203Gln + Gly205Ser + Thr206Gly + Trp212Ala + Gly215Pro + Thr217Ser + Ser221Glu
Gly203Ser + Ile208Cys + Leu209Met + Trp212Ser + Gly215Pro + Ser216Glu + Thr223Pro
Gly205Ser + Thr206Gly + Trp212Gly + Thr217Gly + Arg218Glu + Ile220Gly + Gly222Asn
Gly203Ser + Gly205Pro + Thr206Gly + Gly214Gln + Ser216Asp + Ile220Gly + Thr223Ser
Pro204Asn + Trp212Gln + Ile213Thr + Gly215Ser + Ser216Glu + Thr217Asn + Ile220Asn
Gly203Asn + Pro204Gly + Thr206Asn + Ile208Leu + Thr211Pro + Ile220Leu + Gly222Pro
Gly203Asn + Gly205Asn + Thr211Ser + Trp212Met + Ile213Glu + Gly214Asn + Thr223Asn
Pro204Asn + Gly205Pro + Ile208Ser + Thr211Gln + Ile213Gln + Gly215Gln + Arg218Glu
Gly203Gln + Gly205Gln + Thr206Ser + Ile208Val + Trp212Gln + Thr217Pro + Ile220Glu
Pro204Gly + Gly205Pro + Ile208Met + Thr211Ser + Ile213Gly + Gly215Pro + Ser216Glu
Gly203Gln + Gly205Ser + Thr206Ser + Ile208Ala + Ile213Ala + Ile220Met + Thr223Glu
Pro204Gly + Thr206Gln + Leu209Ala + Thr211Gln + Thr217Asn + Arg218Glu + Ile220Pro
Gly203Gln + Pro204Gly + Gly205Ser + Ser207Asp + Leu209Asn + Gly214Ser + Gly215Pro
Gly203Gln + Gly205Gln + Leu209Met + Trp212Thr + Gly214Gln + Ile220Asn + Gly222Glu

Gly203Ser + Thr206Asp + Ile208Leu + Thr211Pro + Gly214Ser + Gly215Ser + Gly222Gln
Pro204Gln + Thr206Gly + Ile208Asn + Trp212Gly + Ile213Pro + Thr217Pro + Arg218Asp
Gly205Asn + Ile208Thr + Thr211Gly + Ile213His + Thr217Gly + Arg218Glu + Thr223Asn
Gly205Asn + Thr206Pro + Ile208Ala + Leu209Ala + Trp212Leu + Gly215Pro + Gly222Asp
Gly203Pro + Thr206Gln + Ile208Asn + Leu209Cys + Gly215Glu + Gly222Pro + Thr223Ser
Pro204Gln + Ile208Ala + Trp212Asn + Ile213Ser + Gly215Ser + Ser219Asp + Ile220Asp
Gly203Ser + Thr211Ser + Trp212Met + Arg218Asp + Ser219Asp + Gly222Asn + Thr223Asn
Gly203Ser + Gly205Pro + Thr206Pro + Ile208Leu + Arg218Glu + Ser219Glu + Ile220Ala
Pro204Gly + Thr211Asn + Gly214Pro + Ser216Asp + Thr217Glu + Ile220Gln + Gly222Pro
Gly203Asn + Gly205Pro + Leu209Val + Thr211Pro + Gly215Asn + Ser216Glu + Thr217Asp
Pro204Gly + Gly205Gln + Thr211Ser + Trp212Met + Gly215Asp + Ser216Glu + Thr217Pro
Pro204Asn + Thr206Gly + Ile208Met + Leu209Asp + Ser219Glu + Ile220His + Thr223Pro
Gly205Gln + Thr206Glu + Ile208Thr + Leu209Thr + Thr211Gln + Trp212Cys + Ser221Asp
Pro204Gly + Thr206Glu + Leu209Ala + Trp212Met + Ile220Val + Ser221Asp + Gly222Ser
Gly205Gln + Thr206Glu + Thr211Asn + Trp212Ser + Thr217Gln + Ser221Asp + Thr223Gln
Ser207Glu + Leu209Thr + Ile213Gln + Gly214Gln + Gly215Asn + Ser219Glu + Ile220Glu
Thr206Gln + Ser207Glu + Leu209Ile + Ile213Ala + Gly214Ser + Ile220Thr + Ser221Asp
Pro204Asn + Thr206Gln + Ser207Glu + Ile208Ala + Leu209Cys + Thr211Gly + Ile220Glu
Thr206Pro + Ser207Glu + Thr211Gln + Gly214Pro + Gly215Ser + Thr217Gly + Ser219Glu
Pro204Asn + Ser207Glu + Leu209Gly + Thr211Asn + Gly215Asn + Ser219Glu + Ile220Val
Pro204Gln + Gly205Ser + Leu209Ser + Trp212Gly + Thr217Glu + Arg218Glu + Ser219Asp
Gly203Gln + Ser207Asp + Leu209His + Gly214Ser + Gly215Gln + Ser219Asp + Ser221Asp
Pro204Gln + Gly205Asn + Ser207Glu + Leu209Gln + Thr211Gly + Ser219Glu + Ser221Asp
Gly203Asn + Thr206Glu + Ile208Gln + Leu209Gln + Thr211Pro + Trp212Ile + Gly222Glu
Gly203Ser + Thr206Glu + Ser207Asp + Thr211Ser + Trp212Gly + Ile220Gly + Gly222Asp
Gly205Pro + Thr206Asp + Ser207Asp + Ile213Pro + Gly214Ser + Gly222Glu + Thr223Asn
Gly203Gln + Thr206Asp + Leu209Met + Trp212Leu +

Gly214Asn + Gly222Asp + Thr223Glu
Pro204Asn + Thr206Gln + Leu209Ser + Ser216Glu + Thr217Asp + Arg218Glu + Ser219Asp
Pro204Asn + Thr206Gly + Ser207Glu + Ile208Thr + Gly215Gln + Ile220Asp + Gly222Glu
Gly203Pro + Gly205Gln + Thr206Ser + Leu209Asp + Thr211Ser + Ile213Cys + Thr217Asp
Gly205Gln + Ser207Glu + Thr211Ser + Trp212Ser + Ile213Gln + Arg218Asp + Ile220Glu
Gly203Ser + Pro204Gly + Thr206Glu + Ser207Glu + Ile208Ser + Leu209Asp + Trp212Ala
Gly203Ser + Ile208Thr + Leu209Glu + Trp212Tyr + Thr217Gln + Ser219Asp + Ser221Glu
Gly203Ser + Gly205Ser + Thr211Ser + Gly215Gln + Thr217Pro + Ser219Glu + Ser221Asp
Gly205Gln + Thr206Gly + Leu209Ser + Ile213Gly + Ser219Glu + Ser221Asp + Thr223Gln
Pro204Gln + Ser207Asp + Ile208Ser + Ile213Leu + Arg218Glu + Ser219Glu + Ser221Glu
Gly203Asn + Trp212Gln + Gly214Pro + Ser216Glu + Arg218Glu + Ile220Leu + Thr223Pro
Pro204Asn + Gly205Gln + Thr206Gly + Ile213His + Gly214Ser + Ser216Asp + Arg218Glu
Ile208Val + Ile213Met + Gly214Ser + Ser216Asp + Thr217Gly + Arg218Asp + Ile220Met
Pro204Ser + Gly205Asn + Ile208Leu + Leu209His + Ser216Asp + Arg218Glu + Thr223Pro
Gly203Gln + Ile208Cys + Thr211Pro + Trp212Ser + Gly215Ser + Ser216Glu + Arg218Glu
Gly203Pro + Gly205Ser + Leu209Ile + Thr211Asn + Thr217Glu + Ser219Glu + Ile220His
Gly205Ser + Leu209Cys + Thr211Asn + Gly215Gln + Thr217Glu + Ser219Asp + Gly222Pro
Gly203Gln + Ile208Ala + Trp212Ile + Gly215Gln + Thr217Glu + Ser219Asp + Gly222Pro
Gly203Ser + Thr206Glu + Ser207Glu + Ile208Pro + Ile213Thr + Gly222Glu + Thr223Asp
Gly203Ser + Ile208Gln + Trp212Ser + Ile213Leu + Arg218Asp + Ser219Glu + Ser221Asp
Gly203Gln + Gly205Asn + Trp212Asn + Ile213Glu + Ser216Glu + Thr217Pro + Arg218Asp
Thr206Asn + Leu209Asp + Ile213Met + Ser216Glu + Thr217Glu + Ser219Glu + Ile220Gly
Gly205Gln + Thr211Pro + Trp212Val + Gly214Ser + Ser216Asp + Arg218Glu + Ser219Glu
Thr206Asn + Ser207Asp + Thr211Ser + Ile213Cys + Ile220Met + Ser221Asp + Thr223Asp
Gly203Asn + Ile213Ser + Thr217Asp + Arg218Glu + Ser219Asp + Ser221Asp + Thr223Gln
Thr206Asn + Ser207Glu + Leu209Cys + Trp212Ser + Arg218Glu + Ser221Asp + Gly222Pro
Gly203Gln + Ser207Asp + Gly214Pro + Gly215Pro + Arg218Asp + Ser221Asp + Gly222Ser

Gly203Asn + Thr206Glu + Leu209Gln + Arg218Glu + Ser219Asp + Ser221Glu + Gly222Pro
Gly203Pro + Ser207Glu + Trp212Ser + Gly214Pro + Ser219Glu + Ser221Asp + Thr223Asp
Gly205Gln + Thr211Gln + Ile213Glu + Ser216Glu + Arg218Asp + Ser219Asp + Ile220Ala
Gly203Asn + Pro204Ser + Ser207Glu + Gly215Gln + Thr217Asp + Ile220Asp + Ser221Asp
Gly203Gln + Gly205Asn + Ser207Asp + Leu209Ile + Thr211Gly + Thr217Asp + Arg218Glu
Pro204Gly + Trp212Met + Gly214Asp + Ser216Asp + Thr217Glu + Ser219Glu + Thr223Gln
Ser207Asp + Trp212Tyr + Gly215Asn + Ser216Asp + Arg218Glu + Ser219Glu + Thr223Pro
Thr206Gly + Ser207Asp + Ile208Gln + Ser216Glu + Arg218Glu + Ser219Asp + Ile220Gln
Ser207Glu + Leu209Gln + Thr211Pro + Gly215Pro + Ser216Asp + Arg218Glu + Ser219Asp
Gly205Asn + Ser207Asp + Ile208Val + Gly214Pro + Ser216Asp + Arg218Glu + Ser219Glu
Thr206Asp + Ile208His + Thr211Ser + Trp212Asn + Arg218Asp + Ser219Glu + Gly222Pro
Ile208Gly + Trp212Met + Ile213Gln + Ser216Glu + Arg218Asp + Ser219Asp + Ser221Asp
Gly203Asn + Thr206Glu + Leu209Asp + Thr217Pro + Ile220Ser + Gly222Glu + Thr223Asp
Gly203Asn + Thr206Asp + Ile208His + Thr211Ser + Trp212Phe + Arg218Asp + Ser221Glu
Pro204Asn + Gly214Asn + Gly215Pro + Ser216Glu + Thr217Asp + Arg218Asp + Ser221Asp
Pro204Gln + Gly205Pro + Thr211Asn + Gly215Gln + Ser219Glu + Ser221Asp + Thr223Glu
Ser207Asp + Leu209Val + Trp212Cys + Gly214Ser + Arg218Glu + Ile220Glu + Thr223Asp
Thr206Asn + Ser207Asp + Ile208Ala + Gly215Pro + Arg218Glu + Ser221Glu + Thr223Asp
Pro204Gln + Gly205Asn + Thr206Gln + Leu209Cys + Thr217Asp + Arg218Glu + Ser221Glu
Gly203Pro + Thr206Ser + Ile213His + Ser219Glu + Ile220Cys + Gly222Glu + Thr223Asp
Gly205Ser + Thr211Ser + Trp212Gln + Gly214Ser + Ser219Asp + Ile220Glu + Thr223Asp
Ser207Asp + Leu209Gln + Ile213Gly + Ser216Glu + Ser219Asp + Ile220Ser + Ser221Glu
Ser207Asp + Thr211Pro + Trp212Gln + Ile213Pro + Ser216Asp + Ser219Asp + Ser221Asp
Pro204Gly + Gly205Pro + Ile208Gly + Leu209Glu + Ile213Thr + Thr217Asp + Ser221Glu
Thr206Gln + Ser207Asp + Ile208Cys + Leu209Thr + Arg218Asp + Ser219Asp + Thr223Asp
Gly203Pro + Ile208Asn + Thr211Gln + Ile213Met + Thr217Gln + Arg218Glu + Ser221Asp
Gly203Asn + Gly205Asn + Thr206Gln + Thr211Gly +

Trp212Gln + Arg218Glu + Ser221Glu
Gly203Ser + Ile208Pro + Gly215Pro + Arg218Asp + Ile220Asn + Ser221Glu + Thr223Asn
Thr206Pro + Thr211Pro + Gly214Ser + Gly215Pro + Arg218Asp + Ser221Glu + Thr223Ser
Thr206Asp + Ser207Asp + Trp212Thr + Ile213Cys + Ser216Glu + Thr217Pro + Ser219Asp
Gly205Asn + Thr206Asp + Ser207Asp + Ile213Thr + Ser216Glu + Ser219Glu + Ile220Leu
Thr206Ser + Ile213Met + Gly214Asp + Gly215Ser + Thr217Glu + Ser219Asp + Ile220Glu
Pro204Asn + Thr206Pro + Leu209Glu + Thr217Gly + Arg218Glu + Ser219Asp + Thr223Asp
Pro204Gly + Ser207Glu + Thr211Pro + Gly214Asp + Arg218Asp + Ser219Asp + Thr223Gln
Gly205Pro + Leu209Glu + Thr211Asn + Gly214Asp + Thr217Asp + Ile220Met + Gly222Asn
Pro204Ser + Trp212His + Ile213Asn + Gly214Glu + Thr217Glu + Ser219Glu + Gly222Asn
Gly203Gln + Ser207Glu + Ile208Cys + Gly215Pro + Ser219Glu + Ile220Asn + Thr223Glu
Ser207Glu + Ile208Gly + Ile213Pro + Arg218Asp + Ile220Met + Gly222Asp + Thr223Glu
Thr206Asp + Ser207Glu + Ile208Gly + Thr211Gln + Ser216Asp + Ser221Glu + Thr223Asn
Gly203Asn + Ser207Glu + Trp212Ser + Ile213Asp + Gly215Asn + Ser216Glu + Arg218Glu
Gly203Asn + Leu209Ser + Ile213Val + Thr217Pro + Ser219Asp + Ile220Ala + Gly222Glu
Ile208Met + Thr211Gly + Trp212Leu + Gly214Pro + Gly215Pro + Ser219Asp + Gly222Glu
Ser207Glu + Leu209Ser + Ile213Ser + Gly214Asn + Ser216Asp + Arg218Glu + Ser221Asp
Gly203Ser + Ser207Asp + Ile208Asn + Thr211Asn + Gly214Glu + Ser219Asp + Ile220Asp
Ser207Asp + Thr211Asn + Ile213Ala + Gly215Asp + Thr217Ser + Ser219Glu + Ile220Glu
Pro204Ser + Gly205Asn + Thr211Pro + Thr217Glu + Ser221Asp + Gly222Glu + Thr223Glu
Ser207Glu + Leu209Ala + Gly214Pro + Ser216Glu + Thr217Ser + Arg218Glu + Ile220Leu
Ile208Val + Leu209Glu + Trp212Ser + Gly215Glu + Ser219Asp + Ile220Asp + Gly222Pro
Gly203Gln + Ile213Ser + Gly215Glu + Ser216Asp + Ser219Asp + Gly222Ser + Thr223Gln
Gly205Pro + Leu209Val + Gly214Asn + Ser216Glu + Ser219Asp + Ile220Glu + Gly222Asp
Pro204Asn + Leu209Asp + Ile213Met + Gly214Pro + Thr217Glu + Ser221Glu + Gly222Asp
Gly203Ser + Ser207Glu + Trp212Pro + Gly214Asp + Ser216Asp + Thr217Asp + Ile220Met
Thr206Glu + Thr211Gln + Ile213Pro + Gly214Asp + Thr217Gly + Ser221Asp + Gly222Asp

Ile208Cys + Leu209Ser + Thr211Asn + Ile213Ala +
Gly215Asp + Arg218Glu + Ile220Gly
Ile208Thr + Leu209His + Ile213Met + Gly214Glu +
Ser221Glu + Gly222Asp + Thr223Asp
Thr206Gln + Gly214Gln + Gly215Glu + Thr217Gly +
Arg218Glu + Ser219Glu + Ser221Asp
Pro204Ser + Thr206Ser + Ile213Cys + Gly215Glu +
Ser216Glu + Thr217Glu + Thr223Asp
Thr206Gln + Ile208Met + Trp212Gly + Gly214Asp +
Ser216Asp + Thr217Pro + Ser219Asp
Ser207Glu + Ile208Thr + Thr211Gln + Trp212Gln +
Gly215Asp + Ser219Asp + Ser221Asp
Gly203Ser + Pro204Gln + Ser207Glu + Ile208Cys +
Gly214Glu + Ser219Asp + Ser221Glu
Ser207Asp + Ile208Thr + Thr211Asn + Gly215Glu +
Ser219Glu + Ile220Met + Ser221Glu
Pro204Ser + Ser207Glu + Gly214Ser + Gly215Asp +
Ser219Asp + Ile220Met + Ser221Glu
Ser207Asp + Ile208Leu + Trp212Ile + Ile213Asp +
Thr217Asn + Arg218Glu + Ser221Glu
Thr206Glu + Trp212Ser + Ile213Cys + Thr217Gly +
Arg218Glu + Ser219Glu + Thr223Glu
Thr206Asp + Ser207Glu + Trp212Cys + Ile213Cys +
Gly215Asn + Ser216Asp + Arg218Glu
Thr206Glu + Ser207Glu + Gly214Gln + Gly215Pro +
Ser216Asp + Thr217Ser + Arg218Asp
Pro204Ser + Gly205Ser + Trp212Pro + Ser216Asp +
Ser219Asp + Ile220Val + Thr223Ser
Gly203Gln + Leu209Gln + Trp212Ala + Gly215Asn +
Arg218Glu + Ile220Glu + Thr223Glu
Gly203Gln + Leu209Asp + Thr211Asn + Gly214Glu +
Arg218Glu + Ile220Leu + Gly222Ser
Gly203Ser + Pro204Gln + Thr211Pro + Gly215Asp +
Arg218Glu + Ile220Glu + Thr223Asn
Thr206Asn + Ile208Gln + Ser216Glu + Thr217Gly +
Arg218Asp + Ile220Gly + Ser221Asp
Pro204Ser + Ile208His + Gly214Pro + Ser216Glu +
Arg218Glu + Ser221Glu + Thr223Ser
Pro204Gln + Leu209Ile + Trp212Ala + Ser216Asp +
Thr217Ser + Arg218Asp + Ser221Asp
Gly203Pro + Pro204Asn + Ser207Asp + Ile208Cys +
Thr217Gly + Ile220Gly + Thr223Glu
Pro204Ser + Ser207Asp + Leu209His + Thr211Ser +
Gly215Asn + Gly222Ser + Thr223Glu
Ile208His + Thr211Pro + Trp212Leu + Thr217Ser +
Arg218Asp + Ser221Asp + Thr223Glu
Gly205Asn + Thr206Asn + Ser207Asp + Ile213Thr +
Ser216Asp + Ser219Asp + Gly222Glu
Thr206Asp + Ser207Glu + Ile208Leu + Trp212Gly +
Ser216Glu + Thr217Gln + Gly222Asp
Gly205Asn + Thr206Asn + Ser207Asp + Ile208Gln +
Trp212Cys + Ser216Glu + Thr217Glu
Ser207Glu + Ile208Asn + Trp212Thr + Ile213Gln +

Ser216Asp + Thr217Glu + Thr223Gly
Ser207Asp + Ile208His + Thr211Asn + Gly215Asn + Ser216Asp + Thr217Glu + Thr223Gly
Gly203Ser + Pro204Ser + Ile213Thr + Ser216Glu + Thr217Gly + Ser219Asp + Ser221Glu
Gly203Asn + Ile208Ser + Thr211Asn + Ser216Asp + Ser219Glu + Ser221Asp + Thr223Gly
Gly203Ser + Thr206Pro + Thr211Gly + Trp212Thr + Ser216Glu + Ser219Glu + Ser221Asp
Trp212Ser + Gly214Pro + Gly215Asp + Thr217Asp + Ser219Glu + Ser221Asp + Thr223Pro
Thr206Ser + Ser207Asp + Ile208Pro + Leu209Glu + Gly215Asp + Ile220Thr + Ser221Glu
Gly203Asn + Trp212Tyr + Gly214Asp + Gly215Ser + Arg218Glu + Ile220Pro + Thr223Ser
Pro204Ser + Ile208Thr + Thr211Gly + Trp212His + Gly214Asp + Thr217Pro + Arg218Glu
Leu209Glu + Thr211Pro + Ile213Cys + Gly214Glu + Arg218Asp + Ile220Asn + Ser221Asp
Gly205Gln + Thr211Asn + Gly214Asp + Gly215Glu + Thr217Asp + Ile220Ala + Thr223Glu
Gly205Gln + Thr211Gln + Ile213Asn + Gly215Asp + Thr217Glu + Ile220Glu + Ser221Asp
Pro204Asn + Ile213Asp + Thr217Glu + Arg218Glu + Ile220Ser + Gly222Pro + Thr223Glu
Gly205Gln + Ser207Glu + Ile208Cys + Gly214Asp + Ser216Glu + Ser219Asp + Thr223Gly
Gly203Ser + Ser207Glu + Gly215Asn + Ser216Asp + Thr217Gly + Arg218Glu + Gly222Glu
Thr206Gln + Leu209Met + Gly214Glu + Gly215Asn + Ser219Glu + Ser221Asp + Gly222Glu
Leu209Met + Thr211Ser + Trp212Cys + Ser216Glu + Thr217Pro + Ile220Glu + Ser221Glu
Thr206Gly + Thr211Gln + Trp212Cys + Ser216Asp + Ile220Glu + Ser221Asp + Thr223Gln
Gly203Ser + Pro204Asn + Ser207Glu + Ile213Asp + Thr217Ser + Ser219Glu + Gly222Asn
Gly205Pro + Thr206Asp + Trp212Leu + Gly214Asn + Ser216Glu + Ser219Asp + Gly222Ser
Gly203Asn + Pro204Ser + Gly205Gln + Ser207Asp + Trp212Phe + Gly215Asp + Arg218Glu

TABLE 29

Multi-loop Double Mutation Variants

Leu 96Gly + Leu209Pro
Tyr104Cys + Ile220Gln
Leu133Ile + Ser216Glu
Leu 96Ala + Asn162Glu
Gln103Asp + Ile220Thr
Tyr169Ser + Ser190Asp
Val 95Asp + Gly205Gln

Asp 65Glu + Ile213Asn
Gly100Ser + Ile220Glu
Gly100Glu + Asn163Gln
Asn162Ser + Ser207Asp
Ser170Glu + Ile220Ser
Ser140Glu + Ile220Leu
Gln103Glu + Thr106Pro
Leu133Pro + Leu209Asn
Gly203Asn + Gly222Asp
Ser207Glu + Ile213Leu
Ser101Asp + Thr206Gly
Gly203Ser + Ser207Glu
Asn 99Glu + Trp212Asn
Gly102Pro + Ser216Asp
Ser219Asp + Ile220Met
Gly 70Asp + Leu133Ser
Gly136Asn + Ser216Asp
Arg167Glu + Leu209Pro
Asn194Glu + Leu209Gly
Leu209Gln + Ser216Glu
Gly 68Asn + Ser221Glu
Asp 65Glu + Ile107Cys
Arg218Glu + Ile220Ala
Asp 65Glu + Gly135Pro
Gly 68Asp + Phe192His
Gly 66Asn + Leu209Ser
Asn168Ser + Gly214Pro
Thr 71Gly + Leu 96Gly
Gly 70Gln + Ile220Gly
Tyr169His + Leu209Ile
Thr217Pro + Ser219Asp
Gly 70Pro + Ile220Asp
Leu 96Gln + Asn 99Glu
Asp 65Glu + Gly136Ser
Asn 99Glu + Ile213Met
Gly205Pro + Ser207Asp
Asn 99Glu + Leu209Gln
Val 95Gln + Ser207Asp
Val 95Gln + Ser219Asp
Asn 67Ser + Thr217Glu
Gly160Gln + Leu209Asp
Gly136Asn + Gly160Asn
Asn163Gln + Thr223Asn
Leu209Glu + Thr217Asn
Gly102Pro + Trp212Gln
Arg 64Asp + Gly203Asn
Asn 67Gln + Thr211Asn
Asp 65Glu + Tyr137Pro
Gln103Asp + Leu133Ile
Leu 96Glu + Ile213Gln
Asn161Gln + Arg218Glu
Leu209Asn + Gly214Pro
Gly 68Ser + Thr206Asn

Arg167Asp + Phe192Ile
Asp 65Glu + Thr211Ser
Ser207Asp + Thr223Gly
Ser207Asp + Gly222Ser
Tyr104Leu + Arg167Glu
Asp 98Glu + Ile220Asn
Arg 64Glu + Gly215Asn
Asn 67Asp + Asn163Ser
Gly160Ser + Thr206Gln
Ile213Ser + Thr223Asp
Gly215Gln + Ser219Asp
Phe202Leu + Ser219Glu
Ser207Asp + Ile220Cys
Tyr137Ser + Ser219Asp
Gly100Asn + Leu209Thr
Tyr137His + Ser170Asp
Ser170Asp + Ile220Thr
Thr106Gln + Ser207Asp
Arg167Asp + Leu209Pro
Ser105Asp + Gly136Asn
Ser139Asp + Phe192Thr
Ser190Glu + Pro204Ser
Asn 67Glu + Leu209Ala
Trp212Ser + Ser216Glu
Tyr104Ala + Thr206Asp
Asn161Gln + Gly222Asn
Val 95Thr + Leu133Ser
Gly160Ser + Asp165Glu
Asn163Ser + Leu209Val
Gly136Glu + Ile220Pro
Ser191Asp + Pro204Ser
Thr217Glu + Ile220Asn
Leu209Met + Gly214Asp
Thr 71Pro + Ser191Glu
Ser101Glu + Trp212Phe
Gly 70Ser + Ser219Glu
Gly 66Gln + Asn 99Ser
Asn163Gln + Ser190Glu
Ile107Ser + Ser190Asp
Ile208Thr + Ser219Asp
Ser216Asp + Ile220Leu
Ser140Asp + Ala164His
Asp 65Glu + Val 95Ser
Phe202Met + Leu209Ser
Asn 67Gln + Tyr169Gly
Gly136Ser + Asn162Asp
Gly160Pro + Ser219Asp
Gly102Pro + Ser207Asp
Val 95Asn + Ser207Asp
Asp 97Glu + Ile107Gln
Thr206Glu + Ile220Pro
Ser216Glu + Thr223Gly
Asp 97Glu + Leu133Cys

Gly134Gln + Gly135Asp
Thr206Gln + Ile220His
Gly100Asn + Thr223Asp
Gly 66Pro + Ile220Val
Gly 66Asn + Asn168Glu
Ser190Asp + Ile213Val
Gly135Pro + Asn162Glu
Leu133Ile + Gly134Pro
Asn163Ser + Arg167Glu
Gly134Gln + Ser216Glu
Asn194Gln + Leu209Gly
Asn 99Gln + Ile213Met
Leu 96Ile + Gly160Asp
Arg167Glu + Ile220Ala
Val 95Ala + Ser139Asp
Gln103Ser + Gly214Ser
Asn 67Glu + Thr 71Gln
Gly135Pro + Ser191Glu
Ile107Ala + Ile220Glu
Gly214Gln + Ser219Glu
Gly160Asp + Phe192Met
Leu 96Ala + Asp 97Glu
Asn168Gln + Leu209Glu
Gln103Glu + Gly136Asn
Gly215Asp + Gly222Ser
Ser207Asp + Thr223Asn
Ser216Asp + Ile220Cys
Val 95Ser + Thr206Glu
Gly 70Asn + Ser216Asp
Leu209Ser + Ile220Asp
Ala164Pro + Ile208Thr
Tyr137Thr + Leu209Glu
Thr106Asp + Tyr137Thr
Thr106Gly + Asp165Glu
Asn 99Asp + Ile213Gly
Asn161Glu + Ile220Ser
Gly160Asp + Ile220Met
Gly135Asp + Asn163Gln
Tyr169Ser + Ser216Glu
Ser140Asp + Leu209Asn
Ser140Asp + Leu209Gln
Ser170Glu + Gly222Pro
Ile213His + Ser216Asp
Leu 96Thr + Gly222Asn
Asp 65Glu + Asn168Gln
Tyr104Leu + Ser219Glu
Thr106Pro + Gly134Pro
Asn 99Asp + Gly215Asn
Thr206Glu + Ile220Val
Gly 70Pro + Leu 96Glu
Thr106Gly + Ser219Glu
Gly100Glu + Ile107Gly
Gly102Gln + Tyr169Gly

Arg 64Glu + Pro204Gly
Leu133Gln + Ser207Asp
Gly100Gln + Leu133His
Thr106Asp + Leu209Ala
Gly 68Glu + Ile220Gln
Asn 67Asp + Gly 68Gln
Leu 96Glu + Ile220Leu
Thr206Gln + Ser219Asp
Ala164Gln + Gly222Gln
Gly 66Glu + Gly 68Pro
Ser140Asp + Ala166Asn
Asn 67Ser + Gly100Asp
Ser219Asp + Ile220Thr
Ser170Glu + Gly215Ser
Thr 71Gly + Phe192Ile
Gly100Ser + Ser219Asp
Gly215Pro + Ser219Asp
Leu209Met + Ser221Asp
Asn162Asp + Tyr169Met
Tyr104His + Asn162Glu
Asn 67Gln + Leu 96Ser
Leu209Asp + Ile220Ala
Gly 70Ser + Ile220Gln
Gly 66Ser + Gly 70Glu
Tyr169His + Thr206Gly
Asn194Gln + Thr206Gly
Ile107Glu + Leu209Asn
Gly 66Ser + Gly222Glu
Leu133Ile + Ser190Glu
Ser105Asp + Ile220His
Gly102Asp + Asn194Gln
Leu 96Met + Ser216Asp
Asn161Asp + Gly203Gln
Gly136Gln + Asn161Asp
Phe202Ile + Ser219Asp
Gln103Asp + Ile107Thr
Gly100Gln + Ser216Asp
Phe192Ala + Ser207Glu
Gly136Pro + Ser138Asp
Asp 98Glu + Leu209Thr
Asn194Ser + Ser216Glu
Val 95Asp + Gly222Gln
Thr211Pro + Ile220Met
Arg167Glu + Tyr169Ala
Gly 66Asn + Ser138Asp
Asn 67Glu + Leu209Pro
Gly 70Glu + Tyr169Pro
Gly102Pro + Leu209Asn
Gly 68Ser + Ser207Asp
Ser101Glu + Ile220Ser
Tyr137Val + Ser191Glu
Thr211Gly + Arg218Asp
Asn161Gln + Asn194Gln

Thr106Asn + Leu209Gln
Val 95Ser + Gly134Asp
Ser191Asp + Ile220Ser
Asn163Ser + Thr223Glu
Leu209Thr + Arg218Asp
Gly 70Asp + Val 95Gly
Leu209Met + Ser216Asp
Thr206Ser + Arg218Glu
Leu209Ile + Thr217Asp
Ile213Pro + Ser219Glu
Gly205Ser + Thr206Asp
Phe192Gln + Thr206Glu
Gly 68Asp + Leu209Ser
Gly160Gln + Thr206Gly
Gly214Asp + Ile220Leu
Gly 70Asn + Arg218Asp
Gly 70Glu + Gly136Asn
Leu133Glu + Gly203Pro
Gly135Gln + Ser207Glu
Ile107Gly + Ile213Gln
Ser140Asp + Ile220Leu
Ser191Asp + Gly214Gln
Leu209Ser + Gly214Asp
Asn 67Glu + Ile220Gln
Ala166Pro + Ser219Asp
Gly102Ser + Ile220Val
Gln103Ser + Ser140Glu
Gly102Asp + Ile220Gly
Tyr104Ile + Gly134Gln
Ser101Glu + Ile220Cys
Asn162Gln + Ser207Asp
Val 95Pro + Ser207Asp
Asn168Gln + Leu209Ala
Gly100Gln + Gly136Asn
Phe192Pro + Asn194Glu
Asn 99Ser + Thr211Gln
Gly215Pro + Ile220Asp
Arg 64Glu + Leu133Ile
Ser207Glu + Ile220Cys
Asp 97Glu + Thr206Asn
Thr106Asp + Tyr137Met
Ser138Glu + Thr223Pro
Ser138Asp + Ile220Ala
Ser191Asp + Leu209Asn
Leu133Ile + Ser170Glu
Ile107Cys + Ser139Glu
Asn161Gln + Ser207Asp
Thr206Asn + Ser216Glu
Asn 67Gln + Ser207Asp
Leu209Met + Ser219Glu
Gly 68Ser + Asp 97Glu
Gly136Glu + Gly222Asn
Thr106Pro + Leu209Val

Gln103Glu + Ile213Ala
Leu 96Gln + Ser219Glu
Ile107Asp + Ala164Gln
Tyr169Asp + Thr211Pro
Leu133Glu + Leu209Val
Asp 97Glu + Asn161Ser
Ile213Gly + Ser216Asp
Thr206Pro + Ser207Asp
Ala164Gln + Leu209Cys
Ser170Glu + Leu209Ala
Gly160Pro + Leu209Cys
Asn 67Asp + Asn163Gln
Thr206Gly + Ser216Asp
Leu133Thr + Ser216Glu
Asn163Asp + Leu209Thr
Val 95Met + Phe202Ser
Gly136Ser + Gly222Ser
Leu209Met + Ile220Met
Gln103Ser + Asn163Glu
Ile107His + Arg218Asp
Phe192Ala + Gly215Gln
Leu209Thr + Ser216Glu
Tyr169Glu + Thr217Ser
Ser101Asp + Leu209Ala
Leu209Ala + Ser221Asp
Gly 66Asp + Thr106Gln
Val 95Glu + Ile213Cys
Ser105Asp + Thr211Gln
Asp165Glu + Asn168Ser
Gly 66Asn + Ile220Thr
Ser219Glu + Ile220Pro
Gly 68Glu + Ile220Met
Val 95His + Leu209Met
Gly 66Ser + Leu209Met
Ser140Glu + Gly214Gln
Gly160Asn + Leu209Asp
Thr206Asp + Ile220Met
Gly102Asn + Thr206Glu
Gly134Pro + Phe192Pro
Leu133Asp + Phe202Ser
Asn162Glu + Ile220His
Leu133Gly + Leu209Met
Asn194Ser + Ser219Asp
Gly 70Gln + Thr217Pro
Asp 97Glu + Thr217Gln
Gly100Asp + Leu209Cys
Ser216Asp + Ile220Thr
Arg167Glu + Asn194Ser
Gly 66Gln + Ser170Glu
Ser101Asp + Tyr137Ala
Asn 67Gln + Asn161Asp
Asn168Asp + Leu209Ile
Asn 67Gln + Gly 68Glu

Asn161Asp + Thr217Pro
Thr206Asp + Leu209Met
Asp 97Glu + Leu209Met
Asp 97Glu + Tyr104His
Asp 98Glu + Gly102Pro
Tyr104Ala + Ser191Glu
Gly100Ser + Arg167Glu
Ser207Asp + Leu209Ser
Asn194Ser + Ser207Asp
Leu 96Val + Ile220Cys
Asn163Gln + Ser191Asp
Asn162Gln + Asp165Glu
Gly 66Gln + Thr 71Asn
Tyr137Leu + Phe192Asn
Gly 66Asn + Ser207Glu
Asp 97Glu + Leu133Ser
Thr206Gln + Ser216Asp
Leu 96Pro + Ile213Glu
Asp165Glu + Gly214Asn
Ser190Glu + Thr217Gly
Tyr104Met + Ile220Glu
Asp 65Glu + Thr223Ser
Gly100Asn + Ser170Glu
Gly134Ser + Leu209Cys
Ala164Gly + Ser207Asp
Thr 71Asn + Ile220Asp
Ile107Cys + Ser219Asp
Thr106Gly + Asn162Asp
Asn161Glu + Leu209Thr
Gly214Gln + Ser221Asp
Ala164Gln + Ser207Glu
Asn 67Ser + Ser216Asp
Leu209Ala + Thr217Ser
Ala166Gln + Ser216Asp
Leu209Gln + Ile220Glu

TABLE 33

Multi-loop Triple Mutation Variants

Leu 96Gly + Leu209Pro + Ser216Glu
Arg 64Glu + Tyr104Cys + Ile220Gln
Tyr169Ser + Ser190Asp + Ile220Thr
Gly100Glu + Asn162Ser + Asn163Gln
Gln103Glu + Leu133Pro + Leu209Asn
Gly 70Asp + Leu133Ser + Gly136Asn
Gly 68Asn + Leu209Gln + Ser216Glu
Asp 65Glu + Gly135Pro + Ile220Ala
Gly 66Asn + Asn 67Glu + Leu209Ser
Thr 71Gly + Leu 96Gly + Gly214Pro
Gly 70Gln + Leu209Ile + Ile220Gly
Tyr169His + Thr217Pro + Ser219Asp
Asn 67Ser + Val 95Gln + Thr217Glu

Gly136Asn + Gly160Gln + Leu209Asp
Gly136Glu + Asn163Gln + Thr223Asn
Arg 64Asp + Gly203Asn + Trp212Gln
Gly 68Ser + Leu209Asn + Gly214Pro
Arg167Asp + Phe192Ile + Thr206Asn
Ser207Asp + Gly222Ser + Thr223Gly
Gly160Ser + Asn163Ser + Thr206Gln
Gly100Asn + Tyr137Ser + Ser219Asp
Asn 67Asp + Gly100Ser + Tyr137His
Ser105Asp + Gly136Asn + Phe192Thr
Val 95Thr + Leu133Ser + Asn161Gln
Ser140Glu + Asn163Ser + Leu209Val
Pro204Ser + Thr217Glu + Ile220Asn
Ile107Ser + Ile208Thr + Ser219Asp
Asp 65Glu + Val 95Ser + Leu209Ser
Asn 67Gln + Tyr169Gly + Phe202Met
Gly136Ser + Gly160Pro + Asn162Asp
Val 95Asn + Ile107Gln + Ser207Asp
Gly135Asp + Thr206Gln + Ile220His
Gly 66Pro + Gly100Asn + Ile220Val
Gly135Pro + Asn162Glu + Ile213Val
Leu133Ile + Gly134Pro + Arg167Glu
Gly134Gln + Asn163Ser + Ser216Glu
Asn194Gln + Ser207Glu + Leu209Gly
Arg 64Glu + Asn 99Gln + Ile213Met
Tyr104Ser + Arg167Glu + Ile220Ala
Val 95Ala + Gln103Ser + Ser139Asp
Ile107Ala + Gly214Gln + Ile220Glu
Gln103Glu + Gly136Asn + Asn168Gln
Thr206Pro + Ser216Glu + Ile220Cys
Asn 67Asp + Ala164Pro + Ile208Thr
Asp 65Glu + Asn168Gln + Gly222Asn
Tyr104Leu + Leu209Ser + Ser219Glu
Ile107Pro + Asn194Glu + Thr206Gly
Leu133Asn + Leu209Ile + Ile213Asp
Gly 70Pro + Thr106Gly + Ser219Glu
Asp 65Glu + Gly102Gln + Tyr169Gly
Gly100Gln + Leu133Gln + Gly160Asp
Ala164Ser + Ser216Asp + Thr217Gly
Gly 68Glu + Leu209Ala + Ile220Gln
Gly 66Glu + Ala164Gln + Gly222Gln
Thr 71Gly + Phe192Ile + Gly215Ser
Asn 67Gln + Leu 96Ser + Leu209Asp
Arg 64Glu + Gly 70Ser + Ile220Gln
Gly 66Ser + Tyr169His + Thr206Gly
Ser170Glu + Thr206Gly + Gly215Ser
Gly 66Ser + Ile107Glu + Leu209Asn
Ser105Asp + Asn194Gln + Ile220His
Leu 96Met + Asn161Asp + Gly203Gln
Gly136Gln + Asn161Asp + Phe202Ile
Gly100Gln + Phe192Ala + Ser216Asp
Thr211Pro + Ile220Met + Gly222Gln
Gly 68Ser + Thr106Gln + Ser207Asp

Asn161Gln + Thr211Gly + Arg218Asp
Thr106Asn + Asn194Gln + Leu209Gln
Asp 65Glu + Tyr169Val + Ile220Val
Gly 70Asp + Val 95Gly + Leu209Met
Gly205Ser + Thr206Asp + Ile213Pro
Phe192Gln + Thr206Glu + Leu209Ser
Gly160Gln + Thr206Gly + Gly214Asp
Gly 70Asn + Arg218Asp + Ile220Leu
Gly 70Glu + Gly136Asn + Gly203Pro
Ile107Gly + Ile213Gln + Ser219Asp
Gly102Ser + Ala166Pro + Ile220Val
Tyr104Ile + Gly134Gln + Ile220Cys
Val 95Pro + Asn162Gln + Ser207Asp
Gly100Gln + Gly136Asn + Asn194Glu
Asn 99Ser + Thr211Gln + Ile220Asp
Arg 64Glu + Leu133Ile + Ile220Ser
Asp 97Glu + Thr206Asn + Ile220Cys
Ile107Asn + Thr206Asn + Ser207Glu
Asn 67Gln + Leu 96Gln + Ile213Asp
Gly 68Ser + Leu209Met + Ser219Glu
Tyr169Asp + Leu209Val + Thr211Pro
Thr206Pro + Ile213Gly + Ser216Asp
Ala164Gln + Ser207Asp + Leu209Cys
Asn 67Asp + Gly160Pro + Leu209Cys
Asn163Gln + Thr206Gly + Ser216Asp
Val 95Met + Phe202Ser + Leu209Thr
Gly136Ser + Ile220Met + Gly222Ser
Gln103Ser + Asn163Glu + Leu209Met
Ile107His + Phe192Ala + Arg218Asp
Ser101Asp + Leu209Ala + Thr217Ser
Gly 66Asn + Asp165Glu + Asn168Ser
Gly 66Ser + Leu209Met + Gly214Gln
Gly134Pro + Ser140Glu + Phe192Pro
Leu133Gly + Ser207Glu + Leu209Met
Gly 70Gln + Asn163Asp + Asn194Ser
Gly100Asp + Leu209Cys + Thr217Gln
Gly 66Gln + Ser101Asp + Tyr137Ala
Asn 67Gln + Asn168Asp + Leu209Ile
Asn161Asp + Leu209Met + Thr217Pro
Gly100Ser + Ser207Asp + Leu209Ser
Leu 96Val + Asn194Ser + Ser207Asp
Asn163Gln + Ser191Asp + Ile220Cys
Gly 66Gln + Thr 71Asn + Ser216Asp
Gly 66Asn + Tyr137Leu + Phe192Asn
Asp 97Glu + Leu133Ser + Ile220Met
Leu 96Pro + Thr206Gln + Ile213Glu
Asp165Glu + Gly214Asn + Thr217Gly
Asp 65Glu + Gly100Asn + Thr223Ser
Gly134Ser + Ser170Glu + Leu209Cys
Thr 71Asn + Ile107Cys + Ser219Asp
Thr106Gly + Asn162Asp + Leu209Thr
Asn 67Ser + Ala164Gln + Ser207Glu
Ala166Gln + Leu209Ala + Ser216Asp

Leu 96Gly + Leu133Ser + Ser190Glu
Ser207Asp + Leu209Thr + Ile220Pro
Tyr137Thr + Ser139Glu + Gly215Pro
Thr206Gly + Leu209Asp + Thr211Asn
Thr206Asp + Ile208Val + Ile220Leu
Leu 96Cys + Ser207Glu + Ile220Leu
Ile107Leu + Gly136Glu + Ile220Gly
Thr106Asn + Ser139Glu + Ala164Thr
Ser191Glu + Leu209Ser + Ile220Gly
Ser140Glu + Gly215Ser + Gly222Pro
Leu 96Ser + Ala164Gly + Ile220His
Asp 97Glu + Tyr104Asn + Tyr137Gln
Gly 70Gln + Asp 98Glu + Leu133Thr
Gly 66Ser + Thr 71Gly + Phe202Ser
Ser101Asp + Thr206Gly + Ile213Gly
Tyr169Ile + Ser170Glu + Thr211Gly
Gly160Ser + Ile213Val + Ser219Glu
Leu209Gly + Ile220Gly + Thr223Pro
Thr106Gln + Ala164Gln + Phe192Glu
Asn 99Glu + Leu209Gln + Ile220Met
Gly160Gln + Asn194Glu + Gly215Gln
Ser105Asp + Phe202Pro + Leu209Gln
Gly 66Glu + Leu133Thr + Leu209Asn
Ala164Thr + Leu209Met + Ile220Thr
Thr 71Gly + Gly214Asp + Gly222Asn
Val 95Pro + Thr206Gly + Ile213Asp
Leu 96Asn + Gly100Asn + Asn194Ser
Arg 64Glu + Gly100Asn + Ile220Met
Gly 66Ser + Arg218Asp + Gly222Ser
Asp 65Glu + Asn162Gln + Ile220Thr
Asn 99Asp + Thr206Ser + Ile220Ser
Leu 96Ser + Tyr169His + Ser216Asp
Asp 98Glu + Tyr169His + Gly215Asn
Tyr104Leu + Ser207Asp + Leu209Ser
Asn161Ser + Phe192His + Thr206Pro
Leu 96Ser + Ser170Asp + Leu209Ile
Thr106Gln + Asn163Gln + Ser207Asp
Gly 70Gln + Asn 99Ser + Asn161Ser
Asn 99Ser + Ala164Asp + Thr217Pro
Ser139Glu + Thr206Asn + Ile213Leu
Leu 96Asn + Thr217Asn + Ile220His
Gln103Ser + Leu209Pro + Ser219Glu
Asp 65Glu + Leu 96Cys + Gln103Asn
Asn 67Gln + Ser207Asp + Gly214Asn
Gly 68Pro + Thr211Ser + Thr223Asp
Tyr137Gln + Ser138Glu + Ile220Asn
Asp 65Glu + Phe192Val + Gly215Ser
Thr 71Gln + Gly203Gln + Thr206Asp
Arg 64Asp + Asn 99Ser + Thr106Ser
Gly135Gln + Asn168Ser + Phe192Asp
Leu133Ala + Leu209Ala + Ser219Glu
Ala164Thr + Asn168Ser + Ile213Pro
Ser207Glu + Gly214Gln + Ile220Asn

Phe192Tyr + Leu209Pro + Gly215Gln
Asn163Gln + Thr206Gly + Leu209Asn
Leu 96Pro + Asp 98Glu + Asn163Gln
Ser101Asp + Asn168Gln + Tyr169Val
Gly136Pro + Asn162Glu + Ile220Thr
Leu209Cys + Gly214Gln + Ile220Cys
Arg 64Asp + Leu209Ile + Ile220His
Gly134Pro + Gly135Ser + Ser219Glu
Ala164Thr + Leu209Thr + Ser219Glu
Trp212Phe + Ser219Glu + Ile220Glu
Leu 96Glu + Asp 97Glu + Ile220Leu
Leu 96Glu + Asp 97Glu + Gly222Pro
Asn161Asp + Asn162Glu + Gly214Ser
Gly160Asp + Asn161Glu + Ile220Ser
Tyr137Val + Thr206Glu + Ser207Asp
Gly 68Pro + Arg218Glu + Ser219Glu
Leu209Thr + Arg218Glu + Ser219Asp
Tyr104Gln + Ser216Asp + Thr217Asp
Leu209Glu + Thr217Asn + Ser219Asp
Leu209Asp + Ser219Glu + Ile220Ser
Asn194Gln + Ser207Asp + Ser221Glu
Ala164Gly + Ser207Asp + Ile220Asp
Leu 96Val + Ser207Asp + Ile220Asp
Leu 96Glu + Gly102Glu + Ile213Gln
Gly 68Asn + Ser207Glu + Ser219Glu
Ser207Asp + Ser219Glu + Ile220Cys
Leu 96Gln + Ser207Glu + Ser219Glu
Gly102Pro + Ser207Asp + Ser219Asp
Gln103Ser + Ser207Asp + Ser219Glu
Val 95Gln + Ser207Asp + Ser219Asp
Ser207Asp + Ile213Pro + Ser219Glu
Asp 65Glu + Asn 67Asp + Ala164His
Asp 65Glu + Asn 67Glu + Gly136Ser
Asn162Gln + Asp165Glu + Arg167Glu
Leu209Met + Gly214Asp + Ser216Glu
Ser138Asp + Ser170Asp + Ile220Met
Ser138Glu + Asn168Gln + Ser170Glu
Arg 64Glu + Gly 66Asp + Thr217Pro
Asp165Glu + Asn168Asp + Thr211Asn
Leu 96Glu + Asp 98Glu + Gly222Ser
Thr206Ser + Ser216Asp + Arg218Glu
Ala164Ser + Ser216Glu + Arg218Glu
Asn161Asp + Asn162Asp + Thr223Asp
Leu209Ile + Thr217Asp + Ser219Glu
Ser101Asp + Gln103Asp + Leu133Ile
Asn 67Glu + Asp 97Glu + Leu209Ala
Leu133Ile + Ser190Glu + Gly222Glu
Ser207Asp + Ser219Glu + Gly222Asp
Ala164Asp + Asn168Glu + Leu209Pro
Asp 65Glu + Gly 68Glu + Asp 97Glu
Asn163Ser + Thr206Asp + Ser219Glu
Arg 64Glu + Asn 67Asp + Gly215Asn
Tyr137Asp + Ser139Glu + Arg167Asp

Ser140Asp + Ser170Glu + Gly222Ser
Tyr137Glu + Ser140Asp + Leu209Gln
Arg 64Glu + Asp 97Glu + Tyr104His
Val 95Glu + Ser105Asp + Thr211Gln
Gly100Asn + Asn161Glu + Ser221Asp
Asp 65Glu + Asn 99Glu + Trp212Asn
Asp 65Glu + Asn 99Asp + Thr217Ser
Ser190Glu + Ser207Glu + Ile220Glu
Gly 68Glu + Gly136Pro + Ser216Glu
Val 95Glu + Asp 98Glu + Gly102Pro
Asn162Asp + Asp165Glu + Ser191Glu
Val 95Cys + Ser138Asp + Arg167Glu
Leu209Asp + Ser216Glu + Ile220Ala
Gly100Glu + Gln103Asp + Ile107Gly
Gly100Asp + Gly102Pro + Gln103Glu
Ser216Asp + Ser219Glu + Ile220Thr
Gly102Pro + Ser216Asp + Ser219Asp
Tyr104Gln + Ser216Glu + Ser219Glu
Asn 99Gln + Ser216Glu + Ser219Asp
Thr206Gln + Ser216Asp + Ser219Asp
Ser207Asp + Ser216Asp + Thr217Asp
Gly134Asp + Asn162Glu + Phe192Glu
Gly102Glu + Ser105Asp + Leu133Glu
Ser216Asp + Ser219Asp + Ser221Asp
Asn163Asp + Ser170Glu + Asn194Asp
Tyr137Glu + Asn162Glu + Arg167Glu
Ser105Asp + Gly135Asp + Tyr169Asp
Val 95Asp + Asp 98Glu + Ser105Glu
Gly136Asp + Ser140Asp + Gly203Gln
Ser207Asp + Ser216Glu + Ile220Asp
Thr106Gly + Tyr137Asp + Asp165Glu
Asp 65Glu + Gly100Glu + Gln103Asp
Ser101Glu + Arg167Glu + Asn168Glu
Asn 67Asp + Ser191Asp + Phe192Glu
Asp 98Glu + Asn 99Glu + Ser216Asp
Asp 98Glu + Asn 99Asp + Ser216Asp
Arg 64Glu + Asp 65Glu + Asp165Glu
Gly102Glu + Gln103Glu + Ser221Glu
Leu133Glu + Thr206Asp + Ser207Glu
Ser139Asp + Ser216Asp + Thr217Asp
Asp165Glu + Gly215Asp + Ser216Asp
Asp 97Glu + Leu209Glu + Ser219Glu
Ser191Glu + Leu209Glu + Ser219Glu
Thr106Asp + Leu209Asp + Ser219Asp
Asn 99Glu + Ile213Asp + Ser216Glu
Ser207Glu + Leu209Glu + Ile213Asp
Asn 67Glu + Asp 98Glu + Leu209Asp
Ser101Asp + Ser207Asp + Ser221Asp
Gly135Asp + Ser207Asp + Ser219Glu
Asn162Glu + Ser207Asp + Ser219Glu
Ser139Glu + Ser207Glu + Ser219Glu
Gly134Glu + Ser170Glu + Ser216Asp
Asp165Glu + Arg167Glu + Ser207Asp

Asp165Glu + Arg167Asp + Ser219Asp
Asp 65Glu + Ser138Glu + Ser140Asp
Asp 97Glu + Arg167Glu + Tyr169Asp
Ser207Glu + Ile213Glu + Ser216Glu
Thr 71Pro + Ser191Glu + Ser207Glu
Ser191Asp + Ser207Asp + Gly214Gln
Gly 66Glu + Gly 70Glu + Ser216Glu
Asn163Asp + Ser191Asp + Ile220Glu
Asp 97Glu + Ser207Glu + Leu209Glu
Tyr104Asp + Ser207Glu + Leu209Glu
Ser190Asp + Thr206Glu + Ser216Asp
Asp 97Glu + Leu133Glu + Asn161Ser
Asn168Asp + Ser170Asp + Ser190Asp
Asp 98Glu + Ser221Asp + Thr223Glu
Asn 67Asp + Asp 97Glu + Gly135Glu
Asn 67Asp + Asp 97Glu + Ser219Asp
Ser105Glu + Gly160Asp + Gly222Asp
Asp 98Glu + Gly160Asp + Asn163Glu
Ser139Glu + Ser170Glu + Arg218Asp
Gln103Glu + Ser207Asp + Gly222Glu
Ser207Glu + Ser216Asp + Gly222Asp
Gly135Glu + Gly160Glu + Ser216Glu
Tyr104Glu + Asn163Asp + Ser190Glu
Ser190Glu + Asn194Asp + Ser219Asp
Tyr104Met + Ser190Glu + Ile220Glu
Asn 67Asp + Ser216Asp - Ser219Glu
Leu133Asp + Asn162Glu - Phe202Ser
Asn162Glu + Ser191Asp + Leu209Glu
Asp 65Glu + Leu 96Asp - Asn162Asp
Arg 64Asp + Gly136Glu - Arg167Asp
Asn 67Asp + Gly 68Gln - Ser216Glu
Asn 67Glu + Ser216Glu + Ile220Cys
Ser101Glu + Thr206Asp - Leu209Glu
Asp 65Glu + Leu209Glu + Ser216Glu
Ser101Glu + Ile107Glu + Ser219Glu
Gly 68Asp + Ser170Glu + Ser216Asp
Gly136Asp + Ser139Glu + Ser216Glu
Gly135Asp + Leu209Ser + Thr223Asp
Leu133Glu + Asn161Glu + Gly214Glu
Arg 64Glu + Ser191Glu + Ser221Asp
Asn 67Glu + Gly214Asp + Ser219Glu
Thr106Glu + Ser219Glu + Gly222Glu
Asp 98Glu + Ile220Asp + Thr223Glu
Arg167Asp + Gly215Glu + Arg218Asp
Tyr104Asp + Ser139Glu + Ser219Asp
Ile107Gly + Asn161Asp + Ile220Asp
Gly160Glu + Ser216Asp + Ser219Asp
Asp165Glu + Ser216Glu + Ser219Asp
Asp 98Glu + Ser207Asp + Thr223Asp
Ser139Asp + Asn168Glu + Ser207Glu
Ser105Asp + Ser138Glu + Ile220Glu
Ser105Glu + Ser138Asp + Ser219Asp
Ser105Asp + Ser138Asp + Gly222Asp

Ile213Pro	+	Ser216Glu	+ Ile220Glu
Ser101Glu	+	Gly214Glu	+ Arg218Glu
Gly136Glu	+	Asp165Glu	+ Ser190Asp
Asp 65Glu	+	Leu209Cys	+ Ser216Asp
Ser138Asp	+	Asn161Glu	+ Thr206Glu
Gly136Asp	+	Ser140Asp	+ Ser219Glu
Gly 68Glu	+	Gly134Glu	+ Ser219Asp
Gly 68Asp	+	Asn194Glu	+ Ser219Asp
Gly 68Glu	+	Asn161Glu	+ Ser219Asp
Gly 68Asp	+	Ile107Asp	+ Ser219Glu
Gly 68Glu	+	Ser139Asp	+ Ser219Asp
Asp 98Glu	+	Gly135Asp	+ Ser139Glu
Gly135Glu	+	Ser139Glu	+ Leu209Glu
Ala164Glu	+	Ser190Asp	+ Ser219Glu
Gln103Asp	+	Tyr137Asp	+ Gly160Ser
Gly 70Glu	+	Gly102Asp	+ Tyr169Pro
Gly 70Glu	+	Ser219Glu	+ Gly222Asn
Gly 70Glu	+	Leu209Met	+ Ser221Asp
Ile213Ser	+	Ser219Asp	+ Thr223Asp
Gly135Asp	+	Ser140Glu	+ Gly215Asp
Gly102Glu	+	Ser170Glu	+ Thr206Asn
Gly10[illegible]lu	+	Gly135Asp	+ Thr206Glu
Gly 70Glu	+	Asp165Glu	+ Ile220Asp
Phe192Asp	+	Ser207Asp	+ Ile213Glu
Ala164Glu	+	Arg218Asp	+ Thr223Asp
Gly135Asp	+	Ser190Glu	+ Ile220Glu
Asn 99Glu	+	Leu133Asp	+ Asp165Glu
Asp165Glu	+	Ser219Glu	+ Thr223Asp
Asn 67Glu	+	Asn162Asp	+ Ser216Glu

TABLE 34

Multi-loop Quadruple Mutation Variants

Tyr104Cys	+	Leu133Ile	+ Ser216Glu	+ Ile220Gln
Gly102Asn	+	Gly160Ser	+ Ala164Gly	+ Arg218Asp
Asn 67Glu	+	Thr206Gln	+ Leu209Met	+ Ile220Met
Gln103Glu	+	Thr106Pro	+ Leu133Pro	+ Leu209Asn
Val 95His	+	Gly136Asn	+ Gly160Gln	+ Gly222Glu
Arg 64Asp	+	Gly102Pro	+ Gly203Asn	+ Trp212Gln
Val 95Thr	+	Leu133Ser	+ Asn161Gln	+ Gly222Asn
Gly 66Gln	+	Gly 70Ser	+ Asn 99Ser	+ Ser219Glu
Asp 65Glu	+	Gly 68Asn	+ Tyr169Val	+ Ile220Val
Phe192Gln	+	Gly205Ser	+ Thr206Glu	+ Leu209Ser
Leu133Val	+	Asn162Asp	+ Leu209Val	+ Ile220Ala
Leu 96Thr	+	Leu209Ile	+ Ile220Val	+ Thr223Ser
Gly100Glu	+	Gly102Asn	+ Gly134Ser	+ Ile208Pro
Gly100Ser	+	Asn194Gln	+ Ser207Glu	+ Ile220Thr
Asn 67Gln	+	Ala164Gln	+ Tyr169Asp	+ Ile220Thr
Gly160Ser	+	Ser191Glu	+ Ile220Ser	+ Gly222Ser
Thr206Pro	+	Ser207Asp	+ Leu209Cys	+ Ile213Gly
Val 95Met	+	Gly136Ser	+ Phe202Ser	+ Gly222Ser
Gln103Ser	+	Asn163Glu	+ Leu209Met	+ Ile220Met

Ile107His + Phe192Ala + Gly215Gln + Arg218Asp
Gly 66Asn + Asp165Glu + Asn168Ser + Ile220Thr
Gly100Asn + Ser140Glu + Leu209Met + Gly214Gln
Gly 70Gln + Asn163Asp + Asn194Ser + Thr217Pro
Asn161Ser + Phe192His + Thr206Pro + Ser219Asp
Val 95Cys + Leu 96Ser + Ser138Asp + Leu209Ile
Gly 68Gln + Ser101Asp + Thr106Pro + Ile220Met
Gly100Pro + Gly102Gln + Leu209Cys + Gly215Asp
Gly100Asn + Gln103Ser + Ser207Asp + Leu209Val
Thr106Gln + Ile107Asn + Phe192Val + Thr223Gly
Tyr137Gln + Ser138Asp + Leu209Val + Trp212Asn
Leu133Ala + Asn162Asp + Tyr169Val + Leu209Ile
Gly102Ser + Gly134Gln + Gly136Asn + Ser207Glu
Gly100Gln + Gly134Pro + Gly160Asn + Ser219Glu
Tyr137Met + Asn194Gln + Ser207Asp + Gly215Pro
Ala164Asn + Ser190Asp + Phe202Ser + Ile213Asn
Gly 68Pro + Asp 98Glu + Ala166His + Asn168Ser
Ile107Pro + Gly136Pro + Ser207Glu + Gly222Gln
Arg 64Asp + Tyr169Gly + Phe192Pro + Gly205Ser
Val 95Thr + Tyr104Cys + Gly134Asp + Ile208Asn
Ile107His + Gly135Glu + Phe192Gly + Thr217Pro
Ile107Gly + Ser191Glu + Leu209Met + Thr223Gln
Gly 68Gln + Val 95Ala + Ser207Glu + Leu209Asn
Gly 66Asn + Asp 97Glu + Asn161Ser + Gly214Asn
Tyr137Met + Leu209Ser + Ser216Asp + Ile220Pro
Val 95Asn + Leu209Val + Ser219Asp + Thr223Pro
Gly135Asn + Ser140Glu + Ala164Thr + Ile220Leu
Gly 70Ser + Gly135Asp + Thr217Gln + Ile220Thr
Gly 66Ser + Ser140Asp + Leu209Pro + Ile213Leu
Val 95Asn + Leu 96Ile + Thr217Pro + Ile220Gly
Leu 96Met + Gly203Asn + Ser219Glu + Ile220Gly
Tyr104Cys + Tyr169Ser + Leu209Thr + Ser216Glu
Gly 70Gln + Ser138Asp + Thr206Asn + Ile220Gln
Asn163Gln + Gly214Pro + Ile220Asn + Thr223Glu
Gly 68Asn + Ser138Asp + Asn168Ser + Ile220Met
Asn161Gln + Phe192His + Thr217Asp + Ile220Ala
Gln103Ser + Phe192Asn + Arg218Glu + Ile220Thr
Ala164Gly + Asn168Gln + Ser207Asp + Ile208Met
Thr106Gly + Ser139Asp + Phe192Thr + Gly215Asn
Leu209His + Ile213Ser + Gly214Asn + Ser219Glu
Gly 66Asn + Asn 99Ser + Ser101Glu + Asn194Gln
Tyr137Glu + Thr206Pro + Trp212Thr + Ile220His
Gly135Glu + Tyr169Cys + Thr206Gln + Ile220Ser
Thr206Pro + Leu209Ser + Ser216Glu + Ile220Val
Gly 66Asn + Gly 68Asn + Ser105Asp + Leu209Pro
Ser170Glu + Thr206Gly + Leu209Gln + Ile220Cys
Tyr104Thr + Asn163Ser + Leu209His + Gly222Asn
Thr106Glu + Tyr137Gln + Asn162Ser + Leu209Ala
Leu 96Thr + Ser207Asp + Gly214Ser + Ile220Gly
Asn 99Ser + Phe192Pro + Trp212Val + Ser216Asp
Gly136Ser + Tyr137Asp + Leu209Val + Ile220Gly
Gly 66Ser + Gly203Asn + Ser219Glu + Ile220Met
Ala164Thr + Thr206Asn + Thr217Ser + Ser219Glu

Gly102Pro + Thr106Asn + Asn161Glu + Tyr169Ser
Thr106Gly + Gly134Gln + Thr206Asn + Ser207Glu
Gly 66Asn + Gln103Asn + Thr106Gln + Ile220Gln
Arg 64Asp + Asn 67Gln + Ile107Ala + Asn162Ser
Thr 71Gly + Thr106Ser + Asn163Ser + Ile220Asn
Asn 67Gln + Ala164Ser + Ser207Asp + Ile220Thr
Gly 68Ser + Asn163Gln + Arg167Asp + Ile208Thr
Tyr137Ser + Asn168Glu + Leu209Asn + Ile213Ala
Gly134Ser + Gly135Pro + Ala164His + Asn168Ser
Asn163Gln + Ala164Asp + Leu209Gly + Gly215Pro
Gly102Pro + Gly136Pro + Leu209Ser + Ile220Pro
Tyr104Cys + Gly136Asn + Ala164Asn + Leu209His
Asp 65Glu + Leu 96Val + Gly136Asn + Tyr169Pro
Asn 67Asp + Gly100Pro + Tyr104Leu + Leu209Ala
Val 95His + Gly135Gln + Ser191Glu + Ile220Ala
Asn163Ser + Asn194Ser + Ser207Glu + Leu209Ser
Gly 66Glu + Leu 96Ile + Tyr104Thr + Ile213Ala
Gln103Asn + Ser138Glu + Gly160Ser + Phe202Cys
Gly 68Pro + Thr106Ser + Asn194Asp + Thr211Ser
Gly100Gln + Tyr169Thr + Thr211Pro + Thr217Ser
Gly 66Asn + Val 95Cys + Ser139Asp + Asn194Gln
Gly135Pro + Asn161Glu + Asn162Ser + Leu209Ala
Gly 66Ser + Thr 71Ser + Val 95Thr + Ile107Asn
Asn168Gln + Leu209Pro + Ser219Glu + Ile220Gln
Gly 66Pro + Tyr137Asn + Tyr169Ile + Thr223Asp
Ser101Glu + Ile107Pro + Leu133Cys + Leu209Pro
Asn 67Asp + Gly 68Ser + Asn161Ser + Thr223Ser
Asn 99Gln + Gly102Asn + Ile107Met + Trp212Gly
Tyr137Ala + Asn194Gln + Ile213Asp + Gly214Asn
Leu 96Ala + Leu209Met + Gly222Asn + Thr223Gln
Leu 96Val + Asn162Ser + Asn163Gln + Ser170Glu
Asn 67Ser + Leu133Gln + Leu209Gln + Ser221Asp
Val 95Gln + Ala164Thr + Ser207Glu + Leu209His
Gly100Gln + Asn168Gln + Ser219Asp + Ile220Asp
Asp 97Glu + Asp 98Glu + Ile213Ala + Thr217Gly
Gln103Asn + Gly135Ser + Gly215Glu + Ser216Glu
Asn194Ser + Leu209Gly + Gly215Glu + Ser216Glu
Gly 70Gln + Tyr104Cys + Leu209Glu + Ser219Glu
Ile107Val + Thr206Asp + Ser207Glu + Ser221Glu
Leu133Gly + Ser207Glu + Leu209Met + Ser221Glu
Val 95Thr + Tyr137His + Ser207Glu + Ser219Glu
Gly 68Gln + Asn162Ser + Ser207Asp + Ser219Glu
Tyr137Ala + Ser207Glu + Ile213Pro + Ser219Asp
Gly100Asn + Tyr137Ser + Ser207Asp + Ser219Asp
Tyr137Gly + Ser207Glu + Gly215Asn + Ser219Glu
Asp 65Glu + Asn 67Asp + Phe202Ser + Thr206Gly
Gly160Ser + Asn161Glu + Asn194Glu + Thr217Asn
Asn 67Gln + Ser207Asp + Ser219Asp + Ser221Glu
Gly102Asn + Gly134Gln + Ser138Glu + Ser170Asp
Asp 65Glu + Gly 68Asp + Gly135Pro + Phe192His
Phe192Asn + Thr206Glu + Ser207Glu + Ser219Glu
Gly160Pro + Thr206Asp + Ser207Glu + Ser219Asp
Leu 96Asn + Thr206Glu + Ser207Asp + Gly222Glu

Asp 65Glu + Asp 98Glu + Phe192Leu + Ile213Gln
Gly 66Gln + Asn162Ser + Ser207Glu + Leu209Asp
Gly 66Ser + Gly136Gln + Ser190Asp + Thr206Asp
Asn 67Gln + Ile107His + Asp165Glu + Asn168Asp
Phe202Thr + Ser207Asp + Ile220Glu + Gly222Glu
Asn 67Ser + Val 95Gln + Leu209Asp + Thr217Glu
Asn163Asp + Ser191Asp + Asn194Glu + Ile220Met
Ser207Glu + Leu209Glu + Ile213Met + Ser221Asp
Asn 67Glu + Leu 96Asp + Asp 97Glu + Leu209Ala
Thr206Asp + Leu209Asp + Ser219Asp + Ile220Pro
Ser216Glu + Thr217Glu + Ser219Glu + Gly222Gln
Ser139Glu + Ser170Glu + Phe192His + Leu209Gln
Gly102Asn + Pro204Ser + Ser207Asp + Gly222Glu
Thr106Gln + Ser207Asp + Leu209Ser + Gly222Asp
Gly100Ser + Ser207Asp + Ile220Asn + Gly222Asp
Thr106Asn + Ser190Glu + Ser207Asp + Ser221Glu
Leu209Met + Gly214Asp + Thr217Glu + Ile220Asn
Ala164Gln + Ser207Asp + Arg218Asp + Ile220Val
Tyr169Gly + Leu209Asp + Ser216Glu + Ser219Glu
Arg167Glu + Asn194Asp + Leu209Ser + Ile220Pro
Gly102Pro + Ile213Asp + Arg218Asp + Ser219Glu
Asp 98Glu + Gly102Asp + Asn161Gln + Leu209Ser
Ile107Gly + Gly134Asp + Ser138Asp + Tyr169Ser
Gly 68Glu + Gly134Pro + Phe192Pro + Ser216Glu
Asp 65Glu + Gly214Glu + Ile220Thr + Gly222Pro
Ser190Asp + Ser207Asp + Ile208Met + Ile220Val
Gly134Asn + Phe202His + Arg218Asp + Ser221Asp
Leu 96Ala + Ile213Glu + Ser216Glu + Ser219Asp
Gly160Gln + Ser207Asp + Arg218Glu + Gly222Asp
Gly 70Glu + Ile107His + Leu133Glu + Phe192Thr
Gly136Asp + Ser170Asp + Asn194Asp + Gly222Ser
Tyr104Glu + Leu133Asp + Ile213Ser + Gly215Gln
Gly100Gln + Ser207Glu + Ser216Asp + Ser219Glu
Gly100Asn + Tyr137Ala + Leu209Asp + Ser216Glu
Asn162Ser + Ser216Glu + Ser219Asp + Ile220Asn
Val 95Ser + Leu 96Pro + Ser216Asp + Ser219Asp
Gly102Pro + Ser216Asp + Ser219Asp + Ile220Met
Gly135Glu + Gly136Gln + Arg167Asp + Asn194Glu
Asn 99Gln + Ser207Glu + Ser216Asp + Thr217Asp
Asp 65Glu + Ser101Glu + Gly102Glu + Leu133Thr
Asn163Gln + Ser216Glu + Ser219Asp + Ser221Asp
Gly 66Gln + Asn163Asp + Ser170Glu + Asn194Asp
Gly 66Pro + Gly134Asp + Asn161Glu + Ser191Asp
Leu133Thr + Asn161Asp + Ser219Asp + Ile220Asp
Ser105Asp + Gly135Asp + Gly136Ser + Tyr169Asp
Asn 67Gln + Asn163Glu + Asn194Glu + Ser221Asp
Ser207Glu + Ile213Pro + Ser216Glu + Ile220Glu
Gly 68Asp + Leu 96Glu + Thr206Gly + Gly214Glu
Asn 99Glu + Ser101Glu + Gly136Glu + Asn163Gln
Ser138Glu + Ser139Asp + Asn163Asp + Ile220Met
Ser101Glu + Trp212Phe + Ser219Glu + Ile220Glu
Ala164Glu + Asn168Gln + Ser219Glu + Ile220Asp
Ser101Glu + Gly160Gln + Arg167Glu + Asn168Glu

Arg167Glu + Asn168Glu + Asn194Ser + Ser207Asp
Arg167Glu + Asn168Asp + Asn194Ser + Ile220Glu
Leu 96Thr + Arg167Asp + Asn168Glu + Ser216Glu
Thr106Asp + Leu209Asn + Ser221Asp + Gly222Asp
Asp 65Glu + Tyr137His + Asn161Glu + Asn162Asp
Asn 67Asp + Ser191Asp + Phe192Glu + Pro204Ser
Arg 64Asp + Gly 68Ser + Gly222Asp + Thr223Glu
Ser139Glu + Ser140Glu + Arg218Asp + Thr223Pro
Leu 96Met + Asp 98Glu + Asn 99Asp + Ser219Asp
Arg 64Asp + Asp 65Glu + Gln103Asp + Leu209Ile
Tyr137Glu + Ser138Glu + Ser216Asp + Thr217Asn
Gly136Asp + Thr206Asp + Ser207Glu + Ile220Met
Ser101Asp + Ala164Ser + Thr206Asp + Ser207Glu
Gly 66Gln + Tyr104Asp + Ser105Asp + Ser219Asp
Ser138Asp + Thr217Gln + Arg218Glu + Ser219Asp
Arg 64Asp + Leu209Gln + Ser216Glu + Thr217Glu
Ser105Glu + Leu209Asp + Ser219Glu + Thr223Gln
Ser101Asp + Leu209Asp + Ser219Glu + Ile220Ser
Asn163Asp + Phe192Leu + Asn194Glu + Ser207Asp
Asn163Asp + Asn194Glu + Ser207Glu + Trp212His
Ala164Asp + Asn194Glu + Ser207Asp + Ile220Leu
Ala164Glu + Asn194Glu + Ser207Glu + Ile220Ser
Gly100Asp + Ala164Glu + Asn194Asp + Thr206Ser
Leu 96Pro + Ser207Glu + Ser216Glu + Ser221Asp
Val 95Asp + Phe192Tyr + Ser207Asp + Ser221Asp
Ala164Asp + Ser207Asp + Gly215Gln + Ser221Asp
Asp 65Glu + Leu209Gln + Ser216Asp + Arg218Asp
Ile107Cys + Ser139Asp + Ser207Glu + Ser219Asp
Tyr137Glu + Ser207Asp + Ser219Glu + Ile220Ser
Val 95Gln + Tyr104Asp + Ser207Asp + Ser219Asp
Asn163Glu + Ser207Glu + Ser219Glu + Gly222Ser
Asn168Glu + Ser207Glu + Ser219Glu + Gly222Asn
Gly135Asp + Gly203Ser + Ser207Glu + Ser219Glu
Leu 96Thr + Ser139Glu + Ser207Asp + Ser219Asp
Gly100Glu + Ser207Asp + Ser219Glu + Ile220Cys
Gln103Glu + Tyr104Pro + Gly136Glu + Ile220Asn
Gln103Glu + Ser105Glu + Ser219Asp + Ile220Cys
Gln103Glu + Ser105Asp + Tyr137Thr + Thr206Glu
Gly160Asn + Ser207Asp + Gly214Glu + Ser216Asp
Asp 97Glu + Ser101Asp + Ser207Asp + Ile220Thr
Thr106Asp + Tyr137Met + Leu209Glu + Arg218Glu
Gly136Gln + Ser138Asp + Ser170Glu + Ser216Glu
Gly 70Asp + Leu133Ser + Ser138Asp + Ser170Asp
Asn163Glu + Tyr169Cys + Ser219Asp + Gly222Asp
Arg 64Glu + Gly 66Asp + Asn168Gln + Arg218Asp
Ser105Asp + Gly136Asn + Ser139Asp + Phe192Thr
Tyr137Leu + Ser191Glu + Ser207Glu + Gly222Ser
Ser191Asp + Ser207Asp + Leu209His + Ile220Thr
Asn 99Ser + Ser191Glu + Ser207Asp + Ile220Asn
Gly 66Asp + Asn 99Asp + Thr106Gln + Ser216Asp
Asp 97Glu + Phe202Thr + Arg218Glu + Ile220Asp
Gly 70Gln + Asn 99Asp + Ser207Glu + Leu209Glu
Thr106Glu + Ser207Glu + Leu209Asp + Gly214Gln

Asp 65Glu + Tyr137Pro + Asp165Glu + Asn168Asp
Asn 99Gln + Asp165Glu + Asn168Asp + Gly215Glu
Asn 67Glu + Asn161Glu + Ile220Ala + Gly222Glu
Gly 66Asp + Leu 96Gly + Leu209Glu + Ile220Asp
Asp 98Glu + Leu209Ala + Ser219Glu + Ser221Asp
Asn 67Glu + Ala164Gln + Ser219Glu + Ser221Asp
Gly100Glu + Gly136Asn + Ser216Asp + Arg218Glu
Ile107Asp + Thr206Ser + Ser216Asp + Arg218Glu
Ala164Gly + Asn194Asp + Thr217Asp + Ser219Glu
Asp 98Glu + Asn163Asp + Asp165Glu + Trp212Tyr
Ser139Glu + Gly215Glu + Thr217Asp + Gly222Pro
Ser101Glu + Ser105Glu + Phe192Val + Leu209His
Tyr104Asn + Leu209Glu + Ser216Asp + Gly222Asp
Gly 70Glu + Tyr104Ala + Thr206Asp + Ser219Glu
Leu 96Glu + Asn 99Asp + Ala164Gln + Gly214Glu
Ser139Asp + Asn161Glu + Ala164Glu + Ile220Gly
Asn 67Ser + Gly 70Asp + Leu 96Glu + Ser221Glu
Asn 67Asp + Asp 97Glu + Asn163Ser + Ser221Glu
Ser170Glu + Ser207Asp + Ile220Ser + Gly222Asp
Leu 96Gly + Asn163Asp + Thr206Asp + Ser219Glu
Asn 67Asp + Ser190Asp + Gly215Ser + Gly222Asp
Tyr137Cys + Arg167Asp + Ser170Asp + Ser219Asp
Gly135Glu + Ser138Asp + Gly214Glu + Gly222Ser
Asp 65Glu + Asp 97Glu + Asn194Glu + Gly205Asn
Gly102Ser + Ser105Asp + Leu133Glu + Thr223Asp
Gly 70Asp + Asp 98Glu + Leu209Met + Ser216Asp
Ser138Glu + Ala164Ser + Thr206Glu + Ser219Glu
Gly 68Pro + Asp 97Glu + Thr206Asp + Ser219Asp
Ser105Glu + Ser140Glu + Arg218Glu + Thr223Gln
Asn 99Asp + Gly102Glu + Ile213Val + Ser219Glu
Asn 99Asp + Gly102Asp + Gly136Ser + Ser138Asp
Gly 70Glu + Val 95Glu + Gly100Asn + Gly222Glu
Asn 67Glu + Ser216Glu + Ser219Asp + Ile220Cys
Asn 67Asp + Ala164Pro + Ser216Glu + Ser219Asp
Asn 99Asp + Asn161Glu + Ser190Asp + Gly215Asn
Val 95Asp + Asn161Gln + Ser190Glu + Ser221Asp
Gly 68Asp + Gly100Glu + Gly222Ser + Thr223Glu
Gly134Glu + Tyr169Leu + Ser207Glu + Arg218Glu
Thr106Asp + Ser207Glu + Arg218Asp + Ile220Cys
Arg 64Glu + Asn163Ser + Ser207Asp + Arg218Asp
Ser139Asp + Ser207Asp + Arg218Asp + Ile220Thr
Leu133Asp + Gly134Pro + Asn162Asp + Tyr169Asn
Val 95Asp + Gly134Glu + Asn194Glu + Thr211Gly
Ile107Asn + Ser140Glu + Ser170Glu + Ser219Asp
Tyr137Cys + Ser140Glu + Ser170Asp + Ser219Glu
Tyr137Thr + Ser140Glu + Ser170Glu + Thr206Glu
Tyr137Asn + Gly160Glu + Asp165Glu + Ser216Glu
Asn 99Glu + Gly160Asp + Asp165Glu + Leu209Gln
Asp 97Glu + Tyr104Glu + Leu133Ser + Ile220Leu
Asn 67Glu + Val 95Gly + Pro204Ser + Ser216Asp
Asn 99Asp + Asn162Gln + Ser191Asp + Gly222Glu
Gly100Glu + Thr106Gly + Asn162Asp + Tyr169Glu
Asp 65Glu + Asn 99Asp + Gly135Asn + Gly160Asp

Asp 65Glu + Asn 99Glu + Trp212Asn + Ser219Asp
Gly134Glu + Asn161Asp + Thr217Pro + Ser219Asp
Asp 97Glu + Gln103Glu + Asn163Gln + Ile220Glu
Asn161Glu + Asp165Glu + Ser207Asp + Thr223Gln
Ser140Glu + Ala164His + Thr217Asp + Ile220Asp
Gly 70Ser + Gly102Asp + Leu133Asp + Ser191Asp
Ser101Glu + Ile107Glu + Thr217Pro + Ser219Glu
Tyr137Glu + Asn162Glu + Ser190Asp + Ile213Gln
Gly 68Asp + Ser170Glu + Ser216Asp + Ile220Val
Gln103Asp + Gly135Asp + Leu209Met + Ser216Asp
Tyr104Val + Gly136Glu + Ser139Glu + Ser190Asp
Gly102Pro + Asn161Glu + Asp165Glu + Gly214Glu
Gly 70Glu + Tyr169Cys + Ser190Glu + Ser207Glu
Asn168Glu + Ser190Glu + Pro204Ser + Ser207Glu
Ser190Asp + Ser207Asp + Leu209Ser + Ser216Glu
Asn 67Glu + Ser101Glu + Tyr137Asp + Gly215Gln
Arg 64Glu + Ser191Glu + Ile220His + Ser221Asp
Arg167Asp + Ser191Asp + Ile213Ala + Ser221Glu
Arg167Glu + Thr206Ser + Ser219Asp + Gly222Glu
Ser101Glu + Asn161Gln + Asn162Asp + Ser190Asp
Ser101Asp + Gly136Pro + Ser216Asp + Ser219Glu
Tyr104Gln + Thr106Glu + Ser216Glu + Ser219Glu
Gly 68Pro + Leu133Glu + Ser216Glu + Ser219Glu
Gly160Glu + Leu209Val + Ser216Glu + Ser219Glu
Ser191Glu + Leu209Gln + Ser216Asp + Ser219Asp
Asn 67Gln + Arg167Glu + Ser216Glu + Ser219Asp
Asn 99Gln + Ser170Asp + Ser216Glu + Ser219Asp
Ser138Glu + Asn162Gln + Ser216Glu + Ser219Glu
Gly135Gln + Asn168Glu + Ser216Asp + Ser219Asp
Gly100Glu + Thr206Ser + Ser207Glu + Thr223Glu
Asn163Gln + Gly214Asn + Ser216Glu + Ile220Asp
Gly100Pro + Thr211Asn + Ser216Asp + Ile220Glu
Thr206Pro + Leu209Thr + Ser216Glu + Ile220Asp
Gly102Asp + Gly160Asp + Ser190Glu + Trp212Ser
Leu133Cys + Gly134Asp + Asn162Asp + Ser216Asp
Asp 65Glu + Thr106Gln + Ser216Glu + Gly222Gln
Asp 98Glu + Gly100Ser + Asn168Asp + Ile213Glu
Ser101Asp + Tyr137Ala + Asn161Glu + Thr206Glu
Gly 70Asp + Gly136Glu + Ser140Glu + Ile220Pro
Gly 68Glu + Tyr104Glu + Leu209Val + Ser219Asp
Gly 68Asp + Gly160Gln + Asn194Glu + Ser219Asp
Asn163Gln + Ser190Glu + Ser216Glu + Ile220Glu
Arg 64Asp + Gln103Asn + Leu133Asp + Gly214Glu
Arg 64Glu + Tyr104Ser + Leu209Glu + Gly214Asp
Gly 70Asp + Ser101Asp + Ser140Glu + Ile220Val
Gly 70Asp + Leu 96Thr + Leu209Asn + Ser216Glu
Ile107Asp + Ser138Glu + Ala166Gln + Ser219Asp
Asn161Glu + Ser207Asp + Leu209Ile + Thr217Asp
Gly 68Asn + Asn163Glu + Arg167Glu + Ser219Asp
Asn 99Ser + Gln103Asp + Gly136Glu + Thr206Glu
Gln103Glu + Leu133Gln + Gly136Glu + Ser216Glu
Gly102Asp + Leu133Thr + Ser207Asp + Thr217Glu
Asp 97Glu + Ser207Asp + Thr217Glu + Thr223Asn

Ser101Glu + Ser191Asp + Ser207Glu + Leu209Cys
Ile107Ser + Ser191Glu + Ser207Asp + Ser216Asp
Thr 71Pro + Ser191Glu + Ser207Glu + Ser216Glu
Ala164Gln + Ser191Glu + Ser207Glu + Ser216Asp
Ser170Glu + Asn194Asp + Ser207Glu + Ile220Val
Ser170Asp + Asn194Glu + Trp212Ser + Ser216Glu
Arg 64Glu + Asn 99Glu + Ser170Asp + Leu209Thr
Asp 97Glu + Leu133Glu + Asn161Ser + Ser216Asp
Tyr137Pro + Ser140Asp + Phe192Asp + Ser207Glu
Asn 99Ser + Gln103Glu + Ser170Asp + Ser219Asp
Gln103Glu + Asn163Glu + Ser170Glu + Ile220His
Asp 65Glu + Asn 99Ser + Ser101Glu + Ser191Asp
Ser105Glu + Gly135Asp + Thr206Asp + Thr217Gln
Leu 96Cys + Asn161Glu + Arg167Glu + Arg218Asp
Arg 64Glu + Gly100Glu + Asn161Ser + Leu209Glu
Val 95Met + Asp 97Glu + Ser105Glu + Ile220Asn
Gly 68Glu + Val 95Glu + Asn168Ser + Leu209Ala
Asn 67Glu + Asn161Asp + Ser216Asp + Gly222Asn
Asn 67Asp + Leu133His + Arg167Glu + Ser216Glu
Ser105Asp + Gly160Ser + Ser170Asp + Thr206Glu
Gly 66Pro + Asn162Asp + Ser170Glu + Ser219Glu
Gly100Glu + Gly102Asn + Tyr104Asp + Ser207Asp
Gly100Glu + Tyr104Asp + Gly160Glu + Gly215Gln

TABLE 35

Multi-loop Quintuple Mutation Variants

Leu133Gln + Gly136Glu + Thr206Gln + Leu209Met + Ile220Met
Gly203Asn + Gly205Gln + Ser216Glu + Thr217Gly + Ile220Gly
Val 95His + Ala164Ser + Ser170Glu + Phe192Met + Thr223Gly
Leu133Asn + Asn161Gln + Asn163Gln + Leu209Met + Ile220Leu
Asn 67Gln + Gly136Ser + Asn162Asp + Tyr169Gly + Phe202Met
Ile107Asn + Leu209Gln + Ile213Gly + Ser219Glu + Ile220His
Gly 66Ser + Thr 71Gly + Tyr169His + Phe202Ser + Ile220Asp
Gly135Ser + Ala166Ser + Ser207Glu + Ile213Gly + Gly222Ser
Asp 98Glu + Thr106Pro + Tyr137Cys + Trp212Ile + Ile220Val
Gly134Asn + Ala166Pro + Phe192Met + Thr206Gly + Ser219Glu
Thr106Glu + Gly205Asn + Leu209Cys + Gly215Pro + Ile220Thr
Asn163Gln + Asn168Gln + Phe192Cys + Thr206Gln + Leu209Ser
Leu 96Gly + Ile107Gly + Ser140Asp + Leu209Ala +

Thr223Gly
Asn 67Ser + Val 95His + Gly135Gln + Ser191Glu + Ile220Ala
Ile107Gly + Tyr169Ser + Asn194Ser + Ser207Glu + Leu209Ser
Tyr137His + Leu209Met + Ile220Val + Gly222Asn + Thr223Gln
Val 95Ala + Tyr137Pro + Ala164Glu + Asn168Gln + Thr206Asn
Leu 96Gln + Tyr137Leu + Tyr169His + Leu209Asp + Ile220Pro
Gly 66Pro + Gly 70Asn + Leu133Pro + Phe202Pro + Ser207Glu
Val 95Ala + Leu 96Met + Tyr104Asn + Ile107Ala + Ser216Asp
Gly 70Ser + Ile107Gly + Leu133Met + Phe192His + Asn194Asp
Gly 68Pro + Val 95Ser + Gly100Asn + Tyr137Cys + Ile220Pro
Gly 66Ser + Gly102Gln + Ala164Gln + Ser219Glu + Gly222Asn
Gly135Gln + Ser190Glu + Leu209Cys + Gly215Gln + Ile220Val
Gly 68Pro + Asn163Asp + Phe192Pro + Leu209Gly + Ile220Thr
Asn 67Gln + Gly 70Asn + Ile107Leu + Gly136Asn + Asn162Glu
Gly 70Pro + Ile107His + Ser190Glu + Pro204Asn + Leu209Gln
Leu 96Ile + Asp165Glu + Phe202Gly + Leu209Asn + Thr211Gly
Gly 66Pro + Gly 70Asp + Tyr169Asn + Leu209His + Gly215Ser
Tyr104Met + Asn194Glu + Leu209Cys + Thr217Pro + Ile220Gln
Gly 70Asn + Leu 96Ile + Tyr104His + Asn161Asp + Ile220Gln
Val 95Thr + Gly102Pro + Thr206Ser + Gly214Glu + Gly222Ser
Thr106Gln + Asn163Ser + Ile213Pro + Ser216Glu + Ile220Ser
Leu 96Cys + Gly136Asn + Ser170Glu + Thr206Gln + Ile220Gln
Gly 66Gln + Ser105Glu + Gly135Pro + Gly205Asn + Thr206Ser
Gly102Pro + Tyr104Ile + Thr211Gly + Ser216Glu + Thr223Gln
Tyr104Ala + Gly203Asn + Leu209Gly + Arg218Asp + Ile220Cys
Gly 66Pro + Val 95Thr + Asn161Ser + Asn162Gln + Ile220Cys
Gly102Asn + Gly136Gln + Phe202Met + Ile208Val + Ile213Glu

Gly 66Ser + Gly 68Ser + Gly134Gln + Ala164Gly + Asp165Glu
Gly 66Pro + Ala164Asp + Tyr169Ser + Thr206Gln + Thr217Asn
Leu 96Ile + Gly136Gln + Asn161Ser + Thr206Gln + Ser207Asp
Asn 67Asp + Tyr104Asn + Gly135Gln + Tyr169Ile + Leu209His
Gly 70Pro + Gly136Pro + Ser190Asp + Leu209Ser + Gly214Pro
Asp 65Glu + Tyr137Val + Ala164Asn + Phe192Ala + Thr206Ser
Leu 96Thr + Gln103Ser + Tyr104Asn + Asp165Glu + Ile220His
Gly 68Asp + Gly 70Pro + Asn 99Ser + Gly214Ser + Thr223Ser
Leu 96Ser + Leu133Ala + Tyr137Met + Leu209Ile + Ser221Asp
Gly134Gln + Asn194Gln + Thr206Ser + Leu209Met + Ile213Leu
Gly 70Glu + Leu133Cys + Gly135Gln + Thr206Gly + Gly222Asn
Gly134Ser + Ala166Gln + Tyr169Cys + Thr206Gly + Ser221Asp
Gly160Ser + Thr206Asp + Ile213Gln + Ile220Pro + Thr223Gly
Tyr104Asn + Gly135Pro + Leu209Ile + Ser219Glu + Ile220Val
Asn 67Gln + Thr106Asn + Gly160Glu + Tyr169Asn + Leu209Ala
Val 95Glu + Gly100Pro + Thr206Asn + Leu209Thr + Ile220Ala
Asp 98Glu + Gly102Ser + Ala164Gln + Thr206Ser + Ile220Asn
Gln103Asn + Gly136Pro + Ser138Asp + Leu209Gly + Ile220Gln
Asn 67Gln + Thr106Asn + Gly136Gln + Thr206Ser + Ile220Asn
Asn 67Gln + Gly134Gln + Tyr169Cys + Ser219Asp + Ile220Asp
Ala166His + Thr206Glu + Ser207Asp + Ile213Cys + Ile220His
Gly 70Pro + Ile107Gly + Leu209Gln + Ile220Glu + Ser221Asp
Asn 99Ser + Gly160Ser + Gly215Glu + Ser216Glu + Thr217Gln
Gly102Gln + Ile107Cys + Ala164Ser + Leu209Asp + Ser219Asp
Gly134Asn + Tyr137Gln + Leu209Glu + Ser219Asp + Ile220Met
Gly102Gln + Gly160Gln + Leu209Asp + Arg218Asp + Ser219Glu
Gly 70Asn + Ser207Glu + Leu209Val + Ser219Glu +

Ile220Glu
Ala164Asn + Ser207Asp + Thr217Pro + Ser219Glu + Ile220Asp
Val 95Thr + Ala164Pro + Ser207Glu + Ile213Val + Ser221Asp
Val 95Asn + Gly102Pro + Gly160Pro + Ser207Asp + Ser219Asp
Asn 67Gln + Ser207Glu + Leu209Val + Ser219Asp + Ile220Ala
Asn 67Gln + Gly136Gln + Ser138Glu + Ser140Asp + Leu209Met
Tyr104Asn + Ser138Glu + Ser140Asp + Leu209Thr + Ile220Val
Asp 97Glu + Asn 99Asp + Gly102Gln + Tyr137Ser + Ile220Cys
Asn161Asp + Asn163Gln + Tyr169Ala + Asn194Asp + Leu209Ala
Thr206Glu + Ser207Asp + Leu209His + Ser219Asp + Thr223Gly
Gly102Ser + Gly136Asn + Thr206Glu + Leu209Thr + Gly222Asp
Thr 71Gly + Gly102Asn + Ser219Asp + Ile220Cys + Ser221Glu
Thr106Ser + Gly160Asn + Gly205Asn + Ser219Asp + Ser221Asp
Val 95Gln + Leu133Gln + Gly215Asn + Thr217Asp + Ser219Asp
Leu209Ser + Ser219Glu + Ile220Gly + Ser221Glu + Gly222Asp
Gln103Ser + Gly203Pro + Ser216Glu + Arg218Glu + Ser219Asp
Val 95Ser + Gly135Gln + Ser138Asp + Tyr169Glu + Ile213Thr
Asn 67Gln + Gly160Pro + Ser207Glu + Leu209Cys + Gly222Glu
Gly135Asp + Ser138Glu + Gly203Gln + Ile213Met + Gly222Asn
Asn162Gln + Ser207Asp + Leu209Pro + Ser219Glu + Gly222Asp
Tyr137Asn + Ala164Asp + Arg167Asp + Leu209Ile + Ile220Met
Leu 96Gln + Gly102Asn + Gly135Asp + Asn168Asp + Ile220Ala
Val 95Pro + Asn 99Asp + Gly102Asp + Gly160Gln + Leu209Asn
Asn 99Ser + Ile107Gly + Asn162Gln + Leu209Asp + Ser221Glu
Gly 66Pro + Asn162Asp + Ser191Asp + Thr206Asn + Thr223Asn
Gln103Asp + Tyr104Asn + Ser105Glu + Ser140Asp + Leu209Cys
Leu 96Ser + Gly136Ser + Asn168Glu + Asn194Asp + Thr206Asn

Gly 68Glu + Val 95Ala + Gly136Asn + Gly203Asn + Ser216Asp
Asp 65Glu + Thr106Asn + Gly134Gln + Gly214Asp + Ser216Glu
Gly 68Gln + Val 95Pro + Asn162Gln + Ser190Glu + Ser207Asp
Ala166Thr + Ser190Glu + Ser207Glu + Leu209Gln + Thr217Gly
Gly100Pro + Gly160Gln + Ser190Glu + Ser207Asp + Leu209Pro
Asn 67Glu + Ser101Glu + Tyr104His + Phe192Ser + Leu209Thr
Ser191Glu + Phe202His + Leu209Met + Ser221Asp + Thr223Gln
Gly134Asp + Ser139Asp + Ser140Glu + Ile213Met + Thr223Asn
Tyr137Asn + Tyr169Ile + Thr206Asp + Ser219Asp + Thr223Asp
Tyr104Cys + Leu133Ile + Ser207Glu + Ser216Glu + Arg218Asp
Gly 68Asn + Tyr137Ser + Ser207Glu + Ser216Asp + Ser219Glu
Tyr137Ser + Asn168Ser + Gly214Asp + Ser216Asp + Ser219Glu
Ile107His + Asn162Ser + Ser216Glu + Ser219Asp + Ile220Asn
Asn 67Gln + Asn163Ser + Ser216Asp + Ser219Glu + Thr223Ser
Leu 96Asn + Leu133Pro + Thr206Gln + Ser216Asp + Ser219Asp
Ser191Glu + Thr206Asp + Gly214Asn + Ser219Asp + Thr223Asn
Gly 66Ser + Ser105Glu + Thr106Pro + Ser138Glu + Leu209Ile
Gly136Asp + Gly160Glu + Asn163Ser + Ile220Ser + Gly222Asp
Ile107Met + Asn162Asp + Ser190Asp + Ser221Asp + Gly222Ser
Gly 70Gln + Gly214Ser + Ser216Glu + Ser219Glu + Ser221Glu
Gly136Glu + Asn162Glu + Gly214Ser + Gly215Asn + Ile220Leu
Gly 68Asp + Leu209Thr + Arg218Glu + Ser221Asp + Thr223Pro
Gly100Ser + Phe192Glu + Gly215Asn + Ser219Asp + Ile220Glu
Gly134Gln + Phe192Glu + Ile213Gly + Ser219Asp + Ile220Glu
Thr106Gly + Asn162Ser + Asn194Asp + Thr206Asp + Ser221Asp
Gln103Asn + Asn161Asp + Ser207Asp + Thr217Ser + Ile220Glu
Gly 68Asn + Phe192Asp + Ser207Asp + Leu209Asp +

Ile220Asn
Gly 68Asp + Phe192Tyr + Leu209Gly + Ser219Asp + Ile220Gln
Arg 64Asp + Asp 98Glu + Tyr137Val + Phe192Val + Gly214Asp
Tyr104Gly + Ile107Glu + Gly160Glu + Ser170Glu + Leu209Cys
Val 95Thr + Ile107Gly + Ser191Glu + Ser219Glu + Ile220Asp
Asp 65Glu + Gly 66Asp + Gly100Asn + Gly102Ser + Ser190Glu
Asp 65Glu + Gly 66Asp + Gly134Gln + Ile220Asp + Thr223Gln
Asp 65Glu + Gly 66Asp + Leu209Glu + Ile213Ala + Ile220Ala
Asn 99Gln + Ser190Glu + Ser191Asp + Ser219Glu + Ile220His
Gly 70Asp + Ser101Asp + Tyr137Cys + Gly160Ser + Ile220His
Tyr137Ser + Ser138Asp + Ser139Glu + Ile213Met + Ser216Asp
Gly135Ser + Ser138Glu + Asn194Ser + Ser219Glu + Ile220Glu
Thr 71Pro + Asp 97Glu + Thr106Ser + Ser219Glu + Ile220Glu
Arg 64Asp + Gly 66Gln + Thr206Gly + Ser219Glu + Ile220Asp
Val 95Ala + Gln103Ser + Ser139Asp - Arg167Glu - Ile220Ala
Ser101Asp + Gly102Glu + Ile213Val - Thr217Ser - Thr223Glu
Asn 99Asp + Gly100Glu + Tyr137Thr - Asn163Glu - Gly215Gln
Gly 66Ser + Ile107Glu + Leu209Asn + Ser221Glu + Gly222Glu
Asn 67Glu + Thr 71Gln + Asn161Asp + Asn162Glu + Gly214Ser
Gly100Asp + Ser101Glu + Thr106Gln + Ile213Cys + Ser221Asp
Arg 64Asp + Asp 65Glu + Gly 68Ser + Leu 96Gln + Asn168Asp
Gly160Pro + Thr206Glu + Ser207Glu + Gly215Glu + Gly222Pro
Gly102Ser + Tyr137Asp + Tyr169His + Thr206Glu + Ser207Asp
Gly 70Asp + Asn 99Ser + Tyr104Glu + Ser105Asp + Thr217Gln
Leu 96Glu + Gly135Ser + Thr217Ser + Ile220Glu + Ser221Glu
Asp 97Glu + Asp 98Glu + Gly205Pro + Gly215Ser + Ser219Glu
Gly 68Gln + Ser138Asp + Leu209Ile + Arg218Asp + Ser219Asp

Asn 67Ser + Gly136Asp + Gly215Pro + Ser216Glu + Thr217Asp
Asn 99Gln + Ser101Glu + Asn161Gln + Asn162Asp + Asn163Asp
Tyr137Gln + Tyr169Thr + Ser207Asp + Gly215Asp + Ser216Asp
Leu 96Cys + Ser190Asp + Gly215Glu + Ser216Glu + Ile220Cys
Gly160Pro + Phe192Asn + Ser207Glu + Gly215Asp + Ser216Glu
Thr 71Asn + Phe192Val + Ser207Asp + Gly215Asp + Ser216Asp
Gly134Glu + Asn168Asp + Trp212Ile + Ile220Asn + Thr223Glu
Gln103Asp + Gly134Ser + Ser139Glu + Asn162Ser + Ser170Asp
Gln103Asp + Thr106Ser + Ser138Glu + Leu209Val + Gly215Gln
Asn 67Gln + Gln103Asp + Gly135Gln + Ser138Glu + Leu209Asn
Asp 97Glu + Ile107Gln + Gly136Ser + Ser207Asp + Ser221Asp
Tyr137Ile + Ser138Glu + Asn194Ser + Ser207Asp + Ser221Asp
Gln103Asp + Gly135Asn + Gly136Pro + Ser207Glu + Ile220Glu
Gly102Gln + Gly135Glu + Gly203Ser + Ser207Glu + Ile220Glu
Tyr104Ile + Asn163Asp + Arg167Glu + Tyr169Cys + Ile208Cys
Gly 66Glu + Asp 97Glu + Tyr104Glu + Gly136Gln + Leu209Met
Gly102Ser + Ile107Cys + Arg167Asp + Ser207Asp + Ser219Asp
Gly100Asn + Gly134Asp + Ser207Glu + Gly214Ser + Ser219Asp
Arg 64Asp + Val 95Ser + Thr206Ser + Ser207Asp + Ser219Glu
Asn194Asp + Ser207Glu + Ser219Asp + Ile220Met + Thr223Asn
Ser140Glu + Ser207Glu + Leu209Ala + Gly215Gln + Ser219Glu
Tyr104Ser + Gly160Glu + Ser207Glu + Gly215Ser + Ser219Asp
Asp 65Glu + Thr206Ser + Ser207Glu + Gly215Ser + Ser219Asp
Gln103Ser + Asn168Glu + Pro204Gln + Ser207Glu + Ser219Asp
Leu133Thr + Ser140Glu + Asn161Ser + Ser207Asp + Ser219Glu
Leu 96Pro + Ser140Glu + Ser207Glu + Leu209Thr + Ser219Glu
Asn161Asp + Asn168Gln + Ser170Asp + Ser191Asp +

Phe192Gln
Gln103Glu + Thr106Glu + Leu209Gly + Ile213His + Ser219Glu
Asp 65Glu + Asn 67Asp + Thr106Asn + Asn194Glu + Ile208Asn
Asp 65Glu + Asn 67Glu + Leu 96Ala + Gly102Ser + Tyr169Asp
Asn 99Ser + Asp165Glu + Arg167Asp + Ile213Gly + Ser219Asp
Tyr104Ile + Tyr137Ile + Asp165Glu + Arg167Glu + Ser219Asp
Ser190Glu + Phe192Glu + Leu209Cys + Ser219Glu + Ile220Ala
Ser138Glu + Ser140Glu + Thr206Gly + Ile220His + Ser221Glu
Val 95Pro + Ile107Ser + Ser138Asp + Ser140Glu + Ser219Asp
Asp 65Glu + Gly102Ser + Ser138Glu + Ser140Asp + Ala166Gly
Val 95Cys + Ser101Glu + Ser138Glu + Ser140Asp + Phe192Ser
Asp 97Glu + Asn 99Asp + Ser139Asp + Ile213Thr + Thr223Gly
Asp 97Glu + Asn 99Asp + Ser140Glu + Ala166Gln + Phe192Thr
Leu 96Met + Gly136Glu + Asn161Asp + Asn168Gln + Ile220Pro
Gln103Asp + Ser105Glu + Asn162Ser + Ser207Glu + Thr223Gln
Arg 64Glu + Gln103Glu + Ser105Asp + Asn163Gln + Leu209Cys
Tyr137Ala + Ser170Glu + Phe192Met + Gly214Glu + Ser216Asp
Gly 70Ser + Ser105Glu + Gly214Glu + Ser216Glu + Thr223Asn
Gln103Ser + Asn168Asp + Gly205Ser + Gly214Asp + Ser216Asp
Gly135Glu + Tyr169Glu + Ser207Asp + Leu209Met + Ile220Cys
Asn 99Gln + Gly136Asp + Gly160Ser + Ser170Asp + Ser216Asp
Gly136Asp + Tyr169Ser + Ser170Asp + Ile213Glu + Thr217Asn
Asp 97Glu + Ser101Asp + Ser139Glu + Asn162Ser + Ile220Val
Asp 97Glu + Ser101Glu + Ile213Met + Thr217Gly + Arg218Asp
Thr106Asn + Ile107Pro + Asn194Glu + Ser219Asp + Ser221Glu
Leu 96Met + Asn 99Asp + Ser101Glu + Ser139Asp + Leu209Thr
Asn 99Asp + Ser101Glu + Gln103Ser + Ser139Asp + Ile220Pro

Gly 68Glu - Asp 97Glu + Gly160Asn + Asn163Ser + Ile220Asp
Arg 64Asp - Gly 66Asp + Ile107Gly + Ser207Glu + Ile220Val
Gly 70Gln + Gln103Asp + Leu133Ile + Ser140Asp + Leu209Thr
Asn 67Glu + Gly 70Glu + Asn 99Gln + Tyr104Met + Leu209Asp
Tyr104Gln + Ile107His + Ser191Asp + Thr206Glu + Arg218Asp
Asn161Glu + Ala164Gln + Ile213Asp + Ser216Asp + Thr217Asn
Thr106Gln + Gly136Pro + Thr206Ser + Ser207Glu + Thr217Glu
Asp 65Glu + Leu 96Gly + Asp 98Glu + Thr106Asp + Ile220Gln
Asp 65Glu + Asp 98Glu + Ile107Gly + Gly135Asn + Gly136Asp
Gly136Asp + Ser138Asp + Ser207Glu + Leu209Asn + Gly222Asn
Thr106Gln + Gly136Asp + Ser138Asp + Leu209Cys + Ser216Glu
Val 95Ala + Asp 97Glu + Asn168Ser + Ser207Asp + Leu209Glu
Ile107Glu + Thr206Ser + Ser207Glu + Leu209Glu + Thr211Gly
Gly 70Pro + Leu 96Val + Gly102Glu + Ser207Glu + Leu209Asp
Val 95His + Asn194Asp + Ser207Asp + Leu209Asp + Thr217Asn
Ser140Asp + Ala164Pro + Ser207Asp + Leu209Asp + Ile220Gly
Gln103Asp + Tyr104Gln + Gly160Ser + Asp165Glu + Asn168Asp
Asp 65Glu + Gly 66Ser + Gly 70Glu + Gly136Asn + Ser207Asp
Gly 68Asp + Phe192Asn + Gly205Asn + Thr217Asp + Ser221Asp
Gly134Asp + Gly136Asp + Ser207Glu + Leu209Ile + Ile220Cys
Gly100Asn + Gly136Glu + Gly160Gln + Ser219Asp + Ser221Glu
Gly102Ser + Ser170Glu + Gly215Asn + Ser219Glu + Ser221Glu
Gly 66Ser + Asn163Gln + Arg167Glu + Ser219Glu + Ser221Asp
Gly100Glu + Gly136Asn + Leu209Ser + Ser219Glu + Ser221Asp
Leu133Ala + Gly134Asp + Ile213Cys + Ser219Glu + Ser221Glu
Gly102Asn + Gln103Glu + Thr206Ser + Ser216Glu + Arg218Asp
Asp 98Glu + Thr206Gly + Ile213Ala + Ser216Asp +

Arg218Glu
Asn163Asp + Ile208Asn + Ile213Asn + Ser216Asp +
Arg218Asp
Gly 66Ser + Gly 70Glu + Tyr169His + Thr206Gly +
Ile220Asp
Arg 64Glu + Asn 67Glu + Ile107Gly + Leu209Val +
Ile220Glu
Gln103Glu + Gly134Ser + Ser138Glu + Arg167Asp +
Thr223Asn
Gly100Pro + Tyr104Pro + Leu133Gln + Phe192Asp +
Ser207Glu
Gly 68Asn + Ser138Glu + Asn163Glu + Arg167Asp +
Thr206Gly
Gly 70Ser + Gln103Asp + Asn162Ser + Ser170Asp +
Leu209Met
Asp 98Glu + Gly100Ser + Ser101Asp + Asn161Ser +
Ile220Asp
Tyr137Pro + Ser139Asp + Asn161Glu + Ala164Glu +
Ile220Gly
Gly 66Asp + Asn 99Glu + Tyr104Met + Ser207Glu +
Leu209Pro
Gly 66Asp + Asp 97Glu + Tyr104Gln + Ile208Val +
Leu209Asp
Ile107Asp + Phe192Met + Ser207Asp + Gly222Glu +
Thr223Gln
Arg 64Glu + Leu 96Ala + Ala164Asn + Ser207Glu +
Gly222Asp
Leu 96His + Ser207Glu + Ser216Glu + Thr217Asn +
Gly222Glu
Tyr169His + Ser170Glu + Ser207Asp + Ile220Ser +
Gly222Asp
Gly 68Pro + Leu133Glu + Leu209Asp + Gly214Gln +
Ser221Glu
Ser101Asp + Arg167Asp + Ser170Glu + Gly215Asn +
Thr223Gly
Asp 65Glu + Asp 97Glu + Ser207Asp + Gly222Ser +
Thr223Ser
Asp 65Glu + Asp 97Glu + Asn 99Gln + Gly135Glu +
Thr206Gly
Tyr104Met + Gly160Asp + Ala164Glu + Leu209Asp +
Ile220Thr
Leu 96Ser + Asn162Glu + Asp165Glu + Tyr169Cys +
Arg218Glu
Asp 65Glu + Ile107Thr + Asn162Glu + Asp165Glu +
Thr217Pro
Val 95Glu + Ser101Asp + Asn162Glu + Phe192Thr +
Gly215Gln
Gln103Asn + Tyr104His + Thr206Glu + Ser216Glu +
Thr223Glu
Gly135Asp + Asn168Asp + Tyr169Asn + Ser191Glu +
Gly205Pro
Gly102Glu + Ser105Glu + Asn161Gln + Ser207Glu +
Ile220Met

Leu133Glu + Phe192Pro + Thr206Glu + Thr217Pro + Ser219Glu
Asn 99Asp + Ser105Glu + Leu133Val + Ser140Glu + Gly214Ser
Asn 99Glu + Gly102Asp + Asn162Gln + Phe192Pro + Ser219Asp
Ile107Val + Ser190Glu + Asn194Glu + Leu209Thr + Ser216Glu
Gly100Glu + Leu133Glu + Ala166Thr + Ser216Asp + Thr217Pro
Arg 64Asp + Ala164Gln + Ser191Asp + Thr206Glu + Leu209Cys
Leu 96Val + Gly134Asp + Gly136Pro + Ser191Asp + Thr206Glu
Tyr104Asn + Asp165Glu + Phe192Asp + Ser207Asp + Ile220Gln
Ser101Asp + Ala164Ser + Thr206Pro + Ser207Glu + Arg218Asp
Asp165Glu + Ser207Glu + Leu209Asn + Trp212Gly + Arg218Asp
Gly100Asn + Tyr104Pro + Ser138Glu + Ser207Glu + Arg218Glu
Ser140Glu + Ser170Glu + Thr211Gln + Gly215Asn + Ser221Asp
Gly100Glu + Ser140Asp + Ser170Asp + Thr206Ser + Leu209His
Ser140Glu + Tyr169Pro + Ser170Glu + Arg218Asp + Ile220Pro
Gln103Ser + Tyr104Asp + Leu209Glu + Ser221Asp + Gly222Ser
Asp 97Glu + Ile107Cys + Asn162Asp + Ser191Asp + Leu209Ala
Asp 97Glu + Tyr104Glu + Ile107Gly + Leu133Ser + Ile220Leu
Leu 96Glu + Leu133Asp + Gly134Asn + Gly215Ser + Ser216Glu
Asn 67Glu + Leu 96Glu + Thr106Pro + Ile107Cys + Ser207Glu
Arg 64Glu + Asp 97Glu + Gly102Asn + Asp165Glu + Phe192Tyr
Gly 66Ser + Gly134Asp + Asn161Asp + Ser207Glu + Thr217Gly
Asp 65Glu + Gly 70Asn + Gly100Pro + Leu209Asp + Ser216Asp
Gly 68Glu + Ser101Asp + Leu209Thr + Trp212Ala + Ser219Asp
Thr106Gly + Asn163Asp + Leu209Pro + Ser219Glu + Thr223Glu
Asp 65Glu + Gln103Ser + Gly134Glu + Gly136Asn + Ser138Glu
Gln103Ser + Ile107His + Asn161Asp + Asp165Glu + Ser207Glu
Gly 66Pro + Ser140Glu + Ala164His + Thr217Asp +

Ile220Asp
Arg 64Asp + Ser101Asp + Tyr104Leu + Ile107Glu + Ile220Asn
Arg 64Glu + Val 95Met + Gly136Ser + Ser190Glu + Ile213Asp
Gly135Glu + Gly160Pro + Leu209Gln + Ile220Leu + Thr223Glu
Asp 65Glu + Val 95Gln + Ser170Glu + Phe192Met + Gly214Glu
Gly100Asn + Ser190Asp + Ser207Asp + Leu209Ser + Ser216Glu
Val 95Gly + Arg167Asp + Ser190Asp + Ser207Glu + Ile220Asn
Asn 67Glu + Ser101Glu + Leu133Ser + Asn163Asp + Thr223Ser
Asn 67Glu + Asn 99Ser + Ser101Glu + Tyr104Ala + Ser207Glu
Gly134Asn + Ser138Asp + Arg218Asp + Ile220His + Ser221Glu
Gly 66Gln + Val 95Pro + Ser138Glu + Arg218Glu + Ser221Asp
Asn 67Asp + Thr 71Ser + Gly135Glu + Asn161Asp + Ala164His
Asp 65Glu + Gly 66Pro + Ser191Glu + Ile213Ser + Ser221Glu
Gly 70Pro + Gly134Pro + Ser191Asp + Ser216Asp + Ser221Asp
Gly100Glu + Tyr104Glu + Asn163Gln + Ile213Pro + Ile220Ser
Gly134Glu + Gly136Pro + Asn162Gln + Ser216Asp + Thr223Asp
Thr 71Asn + Ser105Asp + Leu209Met + Ser219Asp + Gly222Glu
Gly 66Asn + Gly102Asn + Tyr169Asp + Ser216Asp + Ser219Asp
Gly136Pro + Asn161Glu + Ser216Glu + Ser219Glu + Ile220Ser
Ser140Glu + Ile208Met + Ser216Glu + Ser219Glu + Ile220Asn
Gly160Asp + Thr206Gln + Gly214Ser + Ser216Glu + Ser219Glu
Asn 99Ser + Ser190Glu + Ser216Glu + Ser219Glu + Ile220His
Ser139Asp + Thr206Gln + Ser216Glu + Ser219Asp + Gly222Asn
Gly102Asn + Ser190Asp + Ser216Glu + Ser219Asp + Gly222Gln
Gln103Ser + Asn163Asp + Trp212Met + Ser216Asp + Ser219Asp
Thr 71Gly + Gly136Glu + Asn194Gln + Ser216Asp + Ser219Glu
Asp 97Glu + Leu209Met + Ser216Glu + Ser219Asp + Ile220Val

Asn 99Gln + Asp165Glu + Leu209Met + Ser216Glu + Ser219Glu
Thr106Glu + Asn168Gln + Leu209Met + Ser216Asp + Ser219Glu
Leu 96Gln + Asn161Gln + Tyr169Asp + Ser216Asp + Ser219Glu
Leu 96Met + Asn161Asp + Gly203Gln + Ser216Asp + Ser219Glu
Asn 99Glu + Thr206Ser + Ser207Glu + Leu209Val + Thr223Glu
Ser207Glu + Leu209Met + Ser216Asp + Ile220Leu + Thr223Asp
Val 95Ala + Gly214Asn + Ser216Asp + Ile220Asp + Gly222Pro
Ser101Asp + Tyr104Gln + Gly136Asn + Gly160Glu + Ser190Asp
Gly 70Asp + Gly135Glu + Arg167Asp + Asn168Gln + Ile220Gln
Asp 65Glu + Leu 96Met + Gly136Glu + Asn162Asp + Leu209Ala
Val 95Asp + Asn 99Glu + Asn194Ser + Gly214Pro + Thr223Asp
Gly136Glu + Ala164Ser + Asp165Glu + Tyr169Pro + Ser216Asp
Arg 64Glu + Gly135Asn + Asn162Gln + Ser191Asp + Gly214Asp
Arg 64Asp + Asn162Gln + Thr206Gln + Ser207Glu + Gly214Glu
Leu 96Ala + Gln103Ser + Asn162Glu + Arg167Asp + Gly215Glu
Asn161Ser + Asn162Glu + Arg167Glu + Thr206Pro + Ser219Asp
Ala164His + Asp165Glu + Tyr169Pro + Ser170Asp + Gly215Glu
Val 95Ala + Gln103Glu + Ser138Asp + Gly160Asn + Ser219Asp
Ile107Glu + Leu133Asn + Ser138Asp + Ala164Gly + Ser191Glu
Ile107Glu + Leu133Ser + Asn163Glu + Arg167Glu + Leu209His
Ser101Glu + Leu209Asp + Gly215Gln + Ile220Asn + Gly222Glu
Ser139Asp + Asn162Gln + Tyr169Gln + Leu209Asp + Gly222Asp
Thr106Glu + Gly136Ser + Ser170Glu + Asn194Glu + Thr206Gln
Tyr104Ala + Leu133Asn + Asn162Asp + Ser207Asp + Thr217Asp
Tyr169Cys + Ser191Glu + Ser207Glu + Ser216Asp + Gly222Gln
Val 95Gln + Tyr104Leu + Tyr137Glu + Ser191Glu + Ser207Glu
Gly 66Asp + Gly100Asn + Ser101Glu + Ser139Glu +

Tyr169Gln
Gly 66Glu + Val 95Ser + Ser101Glu + Asn163Ser + Ser191Asp
Arg 64Glu + Val 95Ala + Asn 99Glu + Ala164Glu + Gly214Ser
Arg 64Glu + Asn 99Asp + Ser105Asp + Gly160Pro + Leu209Gly
Asn 67Asp + Val 95Asp + Gly102Pro + Ser207Glu + Gly214Ser
Ser101Asp + Ser105Asp + Asn162Glu + Tyr169Gln + Ile220Ser
Ser139Asp + Gly160Ser + Ala166Thr + Phe192Glu + Ser207Asp
Val 95Gly + Gly136Pro + Asn161Glu + Arg167Asp + Ser207Asp
Asp 65Glu + Ser101Glu + Gly102Asn + Leu133Thr + Ser170Asp
Gly136Gln + Asn161Asp + Arg167Asp + Asn194Ser + Ser216Glu
Gly135Asp + Ala164Asp + Ser216Glu + Ile220Leu + Gly222Asn
Asp 97Glu + Asn 99Gln + Gln103Asn + Ser105Glu + Ala166Ser
Asn 99Gln + Asn163Glu + Ile213Leu + Ile220His + Ser221Asp
Gly135Glu + Asp165Glu + Phe192Gln + Ser207Glu + Gly215Ser
Asn162Asp + Ser170Glu + Asn194Ser + Leu209Gln + Ser219Glu
Gly100Asp + Thr106Asp + Ala164Glu + Phe192Thr + Asn194Gln
Ser105Glu + Asn162Glu + Leu209His + Ser221Glu + Gly222Gln
Gly 68Asn + Gly135Glu + Gly160Gln + Ser216Glu + Ile220Asp
Asp 97Glu + Ala164Gly + Leu209Met + Ser216Asp + Ile220Glu
Gly 68Asp + Tyr137Asp + Asn194Gln + Leu209Cys + Ser221Asp
Ile107Asp + Gly136Gln + Ser139Asp + Ser216Asp + Ile220Thr
Gly100Pro + Ser101Glu + Gly136Glu + Ser191Asp + Phe192Ala
Gly 70Glu + Gly160Pro + Ala164Thr + Ser170Glu + Ser216Glu
Arg 64Asp + Tyr104His + Asp165Glu + Leu209Gln + Thr223Glu
Leu133Ala + Ser138Asp + Leu209Cys + Thr217Glu + Ser221Asp
Ser140Glu + Gly203Asn + Thr217Glu + Ile220Met + Ser221Asp
Asp 97Glu + Thr106Asn + Gly134Glu + Asp165Glu + Thr211Gln

Gly102Pro + Ser140Asp + Asn194Asp + Gly215Pro + Ser221Asp
Arg167Asp + Leu209Asn + Ser219Asp + Ile220Val + Thr223Glu
Gly 68Asp + Gly102Asp + Gly136Ser + Ser170Asp + Phe192Leu

TABLE 36

Multi-loop Sextuple Mutation Variants

Val 95His + Ala164Ser + Ser170Glu + Phe192Met + Leu209Thr + Ile220Pro
Leu133Asn + Asn161Gln + Asn163Gln + Leu209Met + Ile220Leu + Thr223Gly
Gly134Asn + Tyr169Ala + Thr211Pro + Ser216Asp + Ile220Met + Gly222Gln
Gly136Glu + Gly203Gln + Thr206Pro + Leu209Thr + Gly215Pro + Ile220Met
Gly100Ser + Gly135Asn + Ala164Thr + Gly214Glu + Thr217Gln + Ile220Leu
Thr106Ser + Thr206Pro + Ile208Cys + Leu209Ser + Ser216Asp + Ile220Ser
Gly 68Asn + Leu 96His + Gly102Asn + Leu209Cys + Trp212Ala + Ile220Val
Gly 70Pro + Val 95Gln + Gly102Gln + Asn162Asp + Gly214Gln + Ile220His
Asn 67Gln + Leu 96Val + Leu133Ala + Ala164Thr + Leu209Ala + Ser219Asp
Gly100Asn + Leu133Asp + Asn161Gln + Thr206Gly + Ile213Leu + Gly222Gln
Leu 96Asn + Gly100Gln + Thr106Asn + Ser140Glu + Tyr169His + Ile220Asn
Gly 70Pro + Asn 99Ser + Gly135Gln + Tyr169Gly + Thr206Ser + Ser216Asp
Ser101Glu + Gly102Glu + Tyr169Met + Thr206Asn + Leu209Ile + Ile220Gln
Val 95Ala + Asn168Gln + Gly214Asp + Gly215Glu + Ser216Glu + Ile220Pro
Leu 96Pro + Thr106Gly + Ile107Gln + Asn163Glu + Phe192Glu + Leu209Gly
Asn194Ser + Phe202Gly + Ser207Glu + Arg218Glu + Ser219Asp + Ile220Asp
Asp 65Glu + Asp 97Glu + Asp 98Glu + Asn168Ser + Tyr169Thr + Thr211Ser
Asn 67Gln + Gly215Ser + Ser219Glu + Ile220Pro + Ser221Glu + Gly222Gln
Gly102Asn + Gln103Ser + Tyr169Ile + Thr206Gly + Ser216Glu + Arg218Asp
Asn 67Glu + Gly 70Glu + Leu 96Pro + Asn 99Ser + Asn162Gln + Phe192Pro
Asn 67Asp + Gly 68Gln + Gly 70Asp + Leu133Val + Leu209Gly + Thr217Asn

Gly 68Ser + Leu133Glu + Gly135Asp + Ser170Glu + Ile213Asn + Ile220Ser
Val 95Asp + Asp 97Glu + Gly100Asp + Gly135Gln + Leu209Gln + Thr223Ser
Leu 96Cys + Gly134Asp + Gly160Glu + Asn161Glu + Asn162Glu + Leu209Pro
Asp 65Glu + Leu 96Glu + Asp 97Glu + Asn 99Asp + Ile220Ser + Gly222Pro
Val 95Pro + Leu209Ser + Ser219Glu + Ile220Gly + Ser221Glu + Gly222Asp
Gly 66Asn + Asp 98Glu + Ser101Asp + Tyr104Cys + Asn194Gln + Ile220Asn
Ile107Thr + Tyr137His + Phe202Leu + Ser216Glu + Arg218Asp + Ser219Glu
Gly134Pro + Tyr137Pro + Ser207Glu + Leu209Cys + Ser219Asp + Gly222Glu
Leu133Thr + Asn163Ser + Ser207Asp + Ile213Met + Arg218Asp + Ser221Asp
Arg 64Asp + Asp 98Glu + Thr106Ser + Gly160Asn + Ile213Pro + Ile220Asn
Gly 70Gln + Leu 96Asn + Tyr169Met + Thr206Glu + Leu209Cys + Thr223Asp
Gly 68Pro + Ala164Glu + Arg167Glu + Pro204Asn + Thr206Gln + Ile220Ser
Asn 99Ser + Gly135Asp + Tyr137Met + Ser138Glu + Asn168Asp + Thr211Asn
Gly 68Pro + Tyr104Glu + Ser138Glu + Thr217Ser + Ile220Gln + Thr223Pro
Gly 66Ser + Asn163Gln + Thr206Asp + Leu209Gly + Ser219Asp + Ile220Met
Gly 68Asp + Thr106Pro + Thr206Gly + Leu209Glu + Ser219Glu + Ile220Asp
Tyr137Leu + Tyr169Cys + Phe192Asn + Ser207Asp + Arg218Glu + Ile220Leu
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Gly 68Asp + Gly100Gln + Thr206Pro + Ser219Glu + Ile220Glu + Ser221Glu
Tyr137Asn + Ala164Gln + Ser207Glu + Leu209Glu + Arg218Asp + Gly222Glu
Gly135Pro + Ser191Asp + Asn194Gln + Thr206Glu + Ser207Glu + Ser219Glu
Val 95Asp + Ser105Asp + Gly160Asn + Leu209Gly + Gly215Pro + Ile220Ala
Gly 68Asp + Gly100Ser + Gly134Asn + Ser207Glu + Ser219Glu + Ser221Glu
Asn 67Gln + Gln103Ser + Thr206Glu + Leu209Glu + Ser216Glu + Ser219Asp
Tyr104Ile + Thr206Glu + Ser216Asp + Arg218Asp + Ser219Glu + Thr223Ser
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Asn163Ser + Thr206Asp + Ser207Glu + Leu209Ser +

Ser216Glu + Ser219Asp
Asn161Ser + Thr206Ser + Gly214Asp + Gly215Asp + Thr217Glu + Ile220Glu
Asn 99Ser + Ser138Asp + Ser139Asp + Ser140Asp + Ile213Gly + Ser216Asp
Gly 70Glu + Leu 96Asn + Thr206Asn + Ser207Glu + Ser219Asp + Ser221Asp
Tyr104His + Asn161Asp + Ser170Asp + Phe192His + Asn194Asp + Gly222Pro
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Ser207Asp + Ile208Ala + Leu209Thr + Ser216Asp + Ser219Glu + Ile220Leu
Gly100Glu + Leu133Glu + Gly135Glu + Asn161Glu + Ala164Gly + Leu209Thr
Leu 96Asn + Gly136Ser + Leu209His + Gly214Glu + Ser216Asp + Ser219Glu
Tyr104Leu + Ile107Glu + Tyr137Glu + Ser138Asp + Asn168Gln + Leu209Ile
Tyr137Glu + Phe192Met + Ser207Glu + Ser219Asp + Ser221Glu + Thr223Pro
Tyr137Asp + Asn168Asp + Ser170Glu + Phe192Asn + Ser207Asp + Ile220Asn
Val 95Ser + Gly102Ser + Asn162Gln + Leu209Ser + Ser216Glu + Ser219Asp
Gly 66Ser + Gly102Gln + Ser216Glu + Ser219Asp + Ile220Val + Gly222Asn
Asp 97Glu + Asp 98Glu + Ser101Glu + Gly134Pro + Asn162Glu + Thr206Gln
Val 95Pro + Ser101Asp + Gly135Asn + Ser207Asp + Leu209Asp + Ile220Asp
Asp 98Glu + Gly203Ser + Ser207Glu + Leu209Pro + Arg218Glu + Ser219Asp
Thr106Pro + Gly135Asp + Ser140Asp + Asn162Glu + Ser170Glu + Gly214Gln
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Gly 66Pro + Asn 99Gln + Gly136Asp + Arg218Asp + Ile220Glu + Ser221Asp
Tyr104Asp + Gly134Glu + Gly135Asp + Thr211Ser + Ser219Glu + Ile220Val
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Ser138Glu + Thr206Pro + Leu209Thr + Ser216Glu + Arg218Asp + Ser219Asp
Asp 97Glu + Leu209Gln + Ser216Asp + Arg218Asp + Ser219Glu + Ile220Ala
Gly102Asp + Leu133Thr + Asn163Gln + Ser216Glu + Thr217Glu + Ser219Asp

Asp 98Glu + Asn 99Asp + Gly102Asp + Thr106Ser + Ile107Gln + Ser138Asp
Asn 67Asp + Gly 70Glu + Ser170Asp + Phe192Pro + Leu209Thr + Ile213Glu
Gln103Asp + Gly134Asn + Asn162Glu + Phe192Glu + Ile220Leu + Gly222Asp
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Gly136Asn + Ser191Asp + Thr206Pro + Arg218Asp + Ser219Asp + Gly222Asp
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Gly 66Pro + Asn163Glu + Ala164Gln + Phe192His + Ser219Asp + Ile220Asp
Gly100Gln + Tyr104Pro + Tyr137His + Arg167Asp + Asn168Asp + Ser207Asp
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Val 95His + Gly100Gln + Ser139Glu + Ser140Asp + Ser207Glu + Thr217Ser
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Ser105Asp + Asn194Gln + Thr206Asp + Ser207Asp + Leu209Val + Gly215Ser
Asn163Glu + Phe192Pro + Trp212Val + Ser216Asp + Thr217Asp + Gly222Pro
Val 95Asn + Gln103Asn + Leu209Gln + Gly215Asp +

Ser216Glu + Ser221Asp
Gly 66Gln + Gly134Glu + Ser170Asp + Leu209Thr + Ile220Asp + Gly222Asp
Gly 66Gln + Thr106Glu + Ile107Glu + Asn163Asp + Asn194Glu + Pro204Asn
Gly102Glu + Gln103Asp + Phe192Ile + Ser207Glu + Leu209Pro + Ile220Asp
Arg 64Glu + Asp 65Glu + Ala164Asn + Ser207Glu + Leu209His + Ser219Asp
Arg 64Asp + Asp 65Glu + Gly135Pro + Tyr137Met + Ser207Glu + Ser219Asp
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Ser105Glu + Tyr137Ile + Ala164His + Thr206Asn + Ser207Glu + Ser221Glu
Asn 67Gln + Asp 98Glu + Asn161Gln + Asn168Ser + Ser207Glu + Ile220Asp
Gly134Ser + Ser139Asp + Thr206Asp + Ile213Ala + Arg218Glu + Ser219Asp
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Leu 96Gly + Asp 98Glu + Thr106Asn + Leu209Glu + Ser216Asp + Ser219Asp
Gly 70Glu + Asp 97Glu + Asn 99Glu + Gly135Ser + Asn194Glu + Leu209Thr
Thr106Gly + Arg167Glu + Ser207Glu + Gly214Gln + Gly215Pro + Ser219Glu
Ala164Glu + Thr206Gly + Ser207Asp + Leu209His + Ser219Asp + Ile220Gln
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Gly 66Glu + Thr106Gln + Phe192Asp + Asn194Ser + Thr206Glu + Ser207Asp
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Gly 66Gln + Gly102Asn + Gly136Asp + Ser139Glu + Asn168Asp + Ser219Asp
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Ile107Val + Asn162Asp + Ser191Asp + Ser207Asp + Ser219Glu + Gly222Pro
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Gly 70Ser + Asp 98Glu + Gly100Asp + Asn162Gln + Ser207Glu + Ser219Glu
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Arg 64Glu + Gly 66Glu + Asn162Asp + Tyr169Ser + Leu209His + Gly214Glu

Gly 70Gln + Leu133Asp + Gly136Glu + Asn194Asp + Ile213Cys + Gly222Asp
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Gly 68Asn + Asp 98Glu + Ser207Asp + Leu209Asp + Ile220Asn + Thr223Gln
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Asn 99Gln + Gly160Glu + Tyr169Val + Ser191Asp + Ser216Asp + Gly222Asp
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Thr217Glu + Ile220His
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Gly134Asp + Ser139Asp + Ser140Glu + Thr206Asp + Ile213Met + Thr223Asn
Asp 65Glu + Asp165Glu + Phe192Pro + Leu209Gly + Gly215Glu + Ser216Asp
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Tyr137Cys + Ala164Thr + Ser190Glu + Ile208His + Ser216Glu + Gly222Glu
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Val 95Met + Ser139Glu + Ser140Asp + Ala164Asn + Ser216Asp + Ser219Asp
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Gly102Glu + Tyr137Cys + Thr206Ser + Ser207Glu + Ser216Asp + Ser219Asp
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Asn 67Glu + Ser139Asp + Ile208Val + Leu209Ser + Ser216Asp + Thr217Asp
Arg 64Asp + Val 95His + Gly134Glu + Ser138Glu + Arg167Glu + Leu209Cys
Arg 64Asp + Phe192Val + Thr206Asp + Leu209Ala + Ser219Glu + Gly222Ser
Leu 96Ala + Ser140Asp + Phe202Gln + Thr206Asp + Leu209Ala + Ser219Glu
Leu 96Ile + Gly102Pro + Gly134Asp + Thr206Asp + Leu209Ala + Ser219Asp
Gly102Glu + Ser105Asp + Ile107Cys + Ser140Asp + Asn194Asp + Ile220Ala
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Thr106Asn + Asn162Glu + Asn168Asp + Ser216Glu + Arg218Asp + Ile220Asn
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Asn168Glu + Phe192Ser
Val 95Ala + Asp165Glu + Asn168Ser + Ser207Asp + Leu209Glu + Ser216Asp
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Gly 66Asn + Ser191Asp + Phe192Asn + Gly215Glu + Ser219Glu + Ser221Glu
Gln103Glu + Ile107Asn + Ser140Glu + Ser190Glu + Ser191Glu + Leu209Thr
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Val 95Pro + Leu133Thr + Gly136Glu + Asp165Glu + Ser216Asp + Arg218Glu

Leu133Pro + Gly134Asn + Asn194Asp + Thr206Glu + Gly214Glu + Ser221Asp
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Gly 68Glu + Gly102Gln + Gly135Ser + Ser190Asp + Ser207Asp + Ile220Ala
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Ile213Asp + Gly222Glu
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Gly 66Asn + Gly 68Asp + Tyr104Leu + Ser138Asp + Ser139Asp + Ser207Asp
Asp 65Glu + Gln103Glu + Tyr137Cys + Thr206Asn + Ser219Glu + Ile220Glu
Leu 96Asp + Tyr137Asp + Leu209Thr + Ile213Gly + Ser219Asp + Ile220Glu
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Gly 66Ser + Asp 97Glu + Tyr169Thr + Ser170Asp + Thr217Glu + Arg218Asp
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Tyr137Ile + Ser139Glu + Ser140Glu + Gly160Gln + Leu209Asp + Gly214Asp
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Ser105Asp + Thr106Pro + Gly136Pro + Gly160Glu + Leu209Asp + Ser219Asp
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Tyr104Met + Thr106Ser + Ser139Asp + Thr206Pro + Ser219Asp + Gly222Glu
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Arg 64Asp + Gly160Asp + Ser207Glu + Ile208Gly + Ile213Leu + Ile220Asp
Tyr137Asp + Ser170Asp + Phe192Ser + Ser207Glu + Leu209Pro + Gly214Glu
Tyr104Cys + Gly135Asp + Ser138Asp + Thr206Asp + Gly214Gln + Arg218Glu
Arg 64Asp + Asp 97Glu + Ser139Asp + Asn168Gln + Thr206Asn + Gly214Asp
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Gly 66Asp + Ser105Asp + Ala164Gln + Phe192Ala + Ser207Glu + Ser219Asp
Arg 64Glu + Gly 66Asn + Asn 99Ser + Ser138Glu + Ser207Asp + Ser219Glu
Arg 64Asp + Ile107Thr + Ile213Gly + Ser216Asp + Arg218Asp + Gly222Glu
Gln103Glu + Ile107His + Gly135Pro + Gly136Asp + Arg167Asp + Ser219Glu
Gly 68Glu + Asp 97Glu + Thr106Glu + Phe192Met + Ser207Glu + Ile213Ala
Ala164Glu + Asn168Ser + Ser191Glu + Ser216Asp + Ser219Glu + Ile220Thr
Gly 68Gln + Asn162Glu + Ser190Asp + Leu209Thr + Ser219Asp + Gly222Gln
Val 95Cys + Ile107Asp + Tyr137Ala + Asn194Glu + Gly214Asp + Ser216Glu
Asp 65Glu + Gly 70Gln + Leu209Gly + Ser216Glu + Ser221Asp + Thr223Glu
Ser101Glu + Gly205Gln + Leu209Glu + Ser216Asp + Thr217Ser + Ile220Thr
Tyr137Thr + Ser139Glu + Leu209Asp + Gly215Pro + Ser216Glu + Thr217Gln
Asn 99Glu + Gly136Pro + Ser140Glu + Ser170Glu + Asn194Glu + Ile220Met
Gly 66Gln + Asp 98Glu + Gly100Glu + Ser105Glu + Ile220Asp + Thr223Asn
Asp 98Glu + Gly100Glu + Ser105Asp + Gly136Ser + Asn168Glu + Gly214Pro
Val 95Asn + Asp 97Glu + Ser101Asp + Asp165Glu + Phe192His + Ile220Glu
Gly135Glu + Asn163Asp + Phe192Tyr + Asn194Gln + Ser219Glu + Ser221Glu
Asn 99Glu + Ser101Asp + Ser140Asp + Asn161Gln + Ser190Asp + Gly215Asn
Gly 68Gln + Gly100Asp + Gln103Asp + Tyr137Gly + Gly160Asp + Leu209Cys
Gly100Asp + Gln103Asp + Gly160Gln + Leu209Asn + Trp212Ser + Ser221Glu
Gly135Asp + Ser138Asp + Asn162Gln + Leu209Val + Ser216Glu + Thr223Asp
Arg 64Asp + Gly 66Glu + Leu 96Thr + Thr106Ser + Asn161Glu + Arg218Glu
Tyr137Ser + Asn161Asp + Ser170Asp + Ser190Glu + Leu209Asp + Gly215Gln
Leu133Ala + Ser139Asp + Thr206Gln + Leu209Ala + Ser216Asp + Ser219Glu
Asn 67Ser + Val 95Thr + Ser140Glu + Thr206Pro +

Ser216Glu + Ser219Asp
Val 95Asn + Ser138Glu + Ile208Gln + Leu209Val + Ser216Glu + Ser219Asp
Gly 70Asp + Asn 99Gln + Ser101Glu + Leu133Asn + Leu209Gln + Ser216Asp
Tyr104Gln + Ile107His + Ser138Asp + Ser191Asp + Thr206Glu + Arg218Asp
Thr106Glu + Ser138Glu + Thr206Gly + Ser219Glu + Ile220Thr + Ser221Glu
Tyr137Glu + Asn161Glu + Leu209Thr + Ile213Asp + Ser216Asp + Thr217Asn
Arg 64Glu + Asn 67Glu + Ser170Asp + Trp212His + Ile213Ala + Arg218Glu
Val 95Asp + Ile107Gly + Gly134Asp + Ser138Asp + Asn161Gln + Ser190Glu
Asp 65Glu + Asp 98Glu + Gly160Ser + Asp165Glu + Phe192Gly + Ser219Glu
Asp 97Glu + Leu133His + Gly136Glu + Ser138Asp + Leu209Gly + Ser216Asp
Asp 65Glu + Ser138Glu + Tyr169Met + Ser207Asp + Leu209Glu + Gly215Pro
Leu 96Gln + Asp 97Glu + Ser138Glu + Thr206Pro + Ser207Glu + Leu209Asp
Gly 70Gln + Asp 97Glu + Ser190Asp + Thr206Glu + Ser216Asp + Ile220Asn
Arg 64Glu + Gly 66Pro + Gly 70Ser + Asp165Glu + Tyr169Glu + Ser191Asp
Gly136Asp + Asn163Asp + Phe192Pro + Ser216Glu + Arg218Asp + Ile220Val
Gly 66Asp + Tyr137Gly + Asn162Ser + Ala164Glu + Arg167Asp + Ser216Glu
Gly100Asn + Gly102Asp + Tyr104His + Asp165Glu + Ser216Asp + Arg218Asp
Gly102Glu + Leu133Val + Ser140Glu + Thr206Glu + Ile220Glu + Thr223Pro
Ser105Glu + Leu133Asn + Ala164Asn + Asp165Glu + Thr217Glu + Ser219Asp
Gly 70Asp + Val 95Thr + Gly134Asp + Ser139Glu + Asn161Glu + Asn194Ser
Gly100Ser + Gly135Glu + Ser140Glu + Asn161Asp + Ile213Cys + Arg218Glu
Gly136Asp + Tyr137Gly + Ser140Asp + Asn162Glu + Thr211Gly + Gly214Asp
Val 95Gly + Tyr104Ala + Ser138Glu + Asn163Asp + Arg167Asp + Ser219Asp
Asp 65Glu + Gly 70Asn + Val 95Ser + Ser191Asp + Thr206Glu + Ser216Asp
Tyr104Leu + Leu133Cys + Gly134Asp + Asn162Asp + Tyr169Pro + Ser216Asp
Val 95Glu + Asn 99Asp + Tyr104Ala + Tyr137Ala + Leu209Asp + Ile220Cys
Gly 66Glu + Thr106Ser + Leu209Gly + Gly215Asn + Ser216Asp + Ile220His

Thr 71Pro + Gly102Glu + Gly134Asp + Ser139Asp + Ile220Pro + Ser221Asp
Leu 96Ser + Asp 98Glu + Ser101Asp + Gln103Ser + Gly160Asp + Ser216Glu
Leu 96Cys + Ala164Asn + Tyr169Pro + Thr217Asn + Arg218Asp + Gly222Asp
Tyr104Asn + Gly136Glu + Tyr137His + Ser140Glu + Ser216Glu + Ile220Cys
Asp 98Glu + Gly135Asp + Ser138Glu + Asn163Glu + Phe192Cys + Ile220Ser
Gly 66Glu + Asn 99Glu + Gly102Pro + Thr106Asn + Ser139Glu + Ser221Asp
Gly 68Glu + Tyr104Ala + Ala164Gln + Asn168Asp + Ser219Glu + Thr223Asn
Asn 99Glu + Gly102Pro + Ser140Asp + Ser207Glu + Leu209Asn + Gly222Asp
Ser105Asp + Thr106Gln + Gly160Asp + Ser191Asp + Phe192His + Gly203Gln
Asp 98Glu + Tyr104Thr + Asn162Gln + Arg167Asp + Ser170Glu + Ser216Glu
Asn 99Asp + Gly102Ser + Tyr137Asp + Asp165Glu + Ile220Ala + Thr223Gly
Asp 98Glu + Thr106Pro + Gly135Asp + Ser138Glu + Phe192Asp + Ile220Gln
Arg 64Glu + Val 95Ala + Leu 96Asn + Ala164Glu + Leu209Ala + Gly214Glu
Arg 64Glu + Gly136Asn + Ala166Ser + Leu209His + Gly214Asp + Ser221Glu
Asn 67Asp + Gly102Asn + Ala164Glu + Asn168Glu + Ser207Asp + Ile220Leu
Gly135Glu + Gly136Gln + Ser139Asp + Gly160Asn + Ala164Ser + Thr206Glu
Ser105Asp + Asn163Gln + Thr206Glu + Gly215Glu + Arg218Glu + Ile220Cys
Gly 68Gln + Ser101Asp + Ser139Glu + Asn162Asp + Asp165Glu + Leu209Gln
Gly100Ser + Ser140Asp + Asn162Glu + Asp165Glu + Pro204Gln + Ser207Glu
Thr106Glu + Ile107Ala + Gly136Glu + Ser140Asp + Asn168Gln + Ser219Asp
Arg 64Glu + Val 95Thr + Asp 98Glu + Tyr104Thr + Ser140Glu + Ser191Asp

II. Cleaning Compositions

In another embodiment of the present invention, an effective amount of one or more of the enzyme variants are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent

compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet).

The cleaning compositions also comprise, in addition to the Proteinase K variants described hereinbefore, one or more cleaning composition materials compatible with the protease enzyme. the term "cleaning composition material", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, granule, bar, spray, stick, paste, gel), which materials are also compatible with the Proteinase K variant used in the composition. the specific selection of cleaning composition materials are readily made by considering the surface material to be cleaned, the desired form of the composition for the cleaning condition during use (e.g., through the wash detergent use). The term "compatible", as used herein, means the cleaning composition materials do not reduce the proteolytic activity of the Proteinase K variant to such an extent that the protease is not effective as desired during normal use situations. Specific cleaning composition materials are exemplified in detail hereinafter.

As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning

compositions, denture cleaning compositions and contact lens cleaning compositions.

A. Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzyme variants of the present invention can be used in a variety of detergent compositions where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the conventional C_{11}-C_{18} alkyl benzene sulfonates and primary and random alkyl sulfates, the C_{10}-C_{18} secondary (2,3) alkyl sulfates of the formulas $CH_3(CH_2)x(CHOSO_3)^-M^+)CH_3$ and $CH_3(CH_2)y(CHOSO_3^-M^+)$ CH_2CH_3 wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C_{10}-C_{18} alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C_{10}-C_{18} alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C_{10}-C_{18} alkyl polyglycosides, and their corresponding sulfated polyglycosides, C_{12}-C_{18} alpha-sulfonated fatty acid esters, C_{12}-C_{18} alkyl and alkyl phenol alkoxylates (especially ethoxylates and mixed ethoxy/propoxy), C_{12}-C_{18} betaines and sulfobetaines ("sultaines"), C_{10}-C_{18} amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. (Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.) Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C_{10}-C_{18} N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993, incorporated herein by reference.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other

active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C_{10}-C_{16} alkolamides can be incorporated into the compositions, typically at about 1% to about 10% levels. The C_{10}-C_{14} monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as $MgCl_2$, $MgSO_4$, and the like, can be added at levels of, typically, from about 0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight. Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such compositions, typically at levels of from about 0.001% to about 1% by weight. Various detersive and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates,

perborates and the like, can be used in such compositions, typically at levels from about 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type, various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially polyacrylates and polyasparatates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions. Such enzyme stabilizers include propylene glycol (preferably from about 1% to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

1. Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like. Hard surface cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more of the enzyme variants, such hard surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as 0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%.

Preferably the pH should be in the range of about 8 to 12. Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether, diethyleneglycol monobutyl ether, ethyleneglycol monobutyl ether, ethyleneglycol monohexyl ether, propyleneglycol monobutyl ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol. When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile solvents are typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 7-12

Liquid Hard Surface Cleaning Compositions

	Example No.					
Component	7	8	9	10	11	12
Asn67Ser	0.05	0.50	0.02	0.03	0.10	0.03
Val95His	–	–	-	-	0.20	0.02
Na_2DIDA*						
EDTA**	–	–	2.90	2.90	–	–
Na Citrate	–	–	–	–	2.90	2.90
NaC_{12} Alkyl-benzene sulfonate	1.95	–	1.95	–	1.95	–
NaC_{12} Alkylsulfate	–	2.20	–	2.20	–	2.20
NaC_{12}(ethoxy)*** sulfate	–	2.20	–	2.20	–	2.20
C_{12} Dimethylamine oxide	–	0.50	–	0.50	–	0.50
Na Cumene sulfonate	1.30	–	1.30	–	1.30	–
Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
Water****	balance to 100%					

*Disodium N-diethyleneglycol-N,N-iminodiacetate

**Na_4 ethylenediamine diacetic acid

***Diethyleneglycol monohexyl ether

****All formulas adjusted to pH 7

In Examples 7-10, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Asn 67Ser, with substantially similar results.

In Examples 11-12, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Asn67Ser and Val95His, with substantially similar results.

Examples 13-18

Spray Compositions for Cleaning Hard Surfaces and Removing Household Mildew

	Example No.					
Component	13	14	15	16	17	18
Gly134Asn + Ser140Asp	0.50	0.05	0.60	0.30	0.20	0.30
Leu96Gly + Leu209Pro	-	-	-	-	0.30	0.10
Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
Perfume	0.35	0.35	0.35	0.35	0.35	0.35
Water	balance to 100%					

Product pH is about 7.

In Examples 13-16, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly134Asn + Ser140Asp, with substantially similar results.

In Examples 17-18, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly134Asn + Ser140Asp and Leu96Gly + Leu209Pro, with substantially similar results.

2. Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

Examples 19-24

Dishwashing Composition

Component	Example No. 19	20	21	22	23	24
Ala164Asp	0.05	0.50	0.02	0.40	0.10	0.03
Ser191Asp + Phe192Met + Asn194Gln	-	-	-	-	0.40	0.02
C_{12}-C_{14} N-methyl-glucamide	0.90	0.90	0.90	0.90	0.90	0.90
C_{12} ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
C_{12} ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
C_{12} alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
C_{12} amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
Mg^{++} (as $MgCl_2$)	0.20	0.20	0.20	0.20	0.20	0.20
Ca^{++} (as $CaCl_2$)	0.40	0.40	0.40	0.40	0.40	0.40
Water	balance to 100%					

Product pH is adjusted to 7.

In Examples 19-22, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ala164Asp, with substantially similar results.

In Examples 23-24, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ala164Asp and Ser191Asp + Phe192Met + Asn194Gln, with substantially similar results.

3. Fabric cleaning compositions

In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

a. Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from

about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 25-28

Granular Fabric Cleaning Composition

	Example No.			
Component	25	26	27	28
Ile208Ala	0.10	0.20	0.03	0.05
Trp212Phe	-	-	0.02	0.05
C_{13} linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylaenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ile208Ala, with substantially similar results.

In Examples 27-28, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ile208Ala and Trp212Phe, with substantially similar results.

Examples 29-32

Granular Fabric Cleaning Composition

Component	Example No. 29	30	31	32
Asn194Glu + Leu209Gly	0.10	0.20	0.03	0.05
Gly100Asn + Gln103Ser + Ser207Asp + Leu209Val	-	-	0.02	0.05
C_{12} alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C_{12}-C_{14} secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 29-30, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Asn194Glu + Leu209Gly, with substantially similar results.

In Examples 31-32, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Asn194Glu + Leu209Gly and Gly100Asn + Gln103Ser + Ser207Asp + Leu209Valo, with substantially similar results.

Examples 33-36

Granular Fabric Cleaning Composition

	Example No.			
Component	33	34	35	36
Arg64Asp + Gly70Gln + Thr71Gly	0.10	0.20	0.03	0.05
Phe192Asp + Asn194Ser	-	-	0.02	0.05
C_{13} linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylaenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 33-34, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Arg64Asp + Gly70Gln + Thr71Gly, with substantially similar results.

In Examples 35-36, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Arg64Asp + Gly70Gln + Thr71Gly and Phe192Asp + Asn194Ser, with substantially similar results.

Examples 37-40

Granular Fabric Cleaning Composition

Component	Example No. 37	38	39	40
Ala164His + Ala166Gly	0.10	0.20	0.03	0.05
Ile213Pro + Ser216Glu + Ile220Glu	-	-	0.02	0.05
C_{12} alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C_{12}-C_{14} secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 37-38, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ala164His + Ala166Gly, with substantially similar results.

In Examples 39-40, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ala164His + Ala166Gly and Ile213Pro + Ser216Glu + Ile220Glu, with substantially similar results.

Examples 41-42

Granular Fabric Cleaning Composition

	Example No.	
Component	41	42
Linear alkyl benzene sulphonate	11.4	10.70
Tallow alkyl sulphate	1.80	2.40
C_{14-15} alkyl sulphate	3.00	3.10
C_{14-15} alcohol 7 times ethoxylated	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate	14.00	15.00
Citric acid	3.00	2.50
Zeolite	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta methylene phosphonic acid	1.00	0.20
Gly203Gln + Thr211Gly + Ile213Leu + Gly214Asn	0.30	0.30
Lipase	0.36	0.40
Amylase	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

Examples 43-44

Granular Fabric Cleaning Composition

	Example No.	
Component	43	44
Sodium linear C_{12} alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylethylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Asp98Glu + Tyr104Leu	0.4	0.4
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45

Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C_{25} and C_{45} alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Gly136Ser + Tyr137Met + Ser138Glu	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylethylene diamine	6.0
Percarbonate	22.0

Ethylene diamine disuccinic acid	0.3
Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyril) biphenyl	0.05
Water, Perfume and Minors	Up to 100

Example 46

Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C_{16}-C_{18} alkyl sulfate	1.3
C_{14-15} alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylethylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Ser219Asp	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100

Example 47

Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C_{14-15} alcohol 7 times ethoxylated	4.4
C_{12-15} alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Ser190Glu + Ser191Glu + Phe192Leu + Asn194Ser	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylethylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b. Liquid fabric cleaning compositions

Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 48-52

Liquid Fabric Cleaning Compositions

	Example No.				
Component	48	49	50	51	52
Ile220Asp	0.05	0.03	0.30	0.03	0.10
Thr206Asp + Ile220Met	-	-	-	0.01	0.20
C_{12}- C_{14} alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C_{10} alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 48-50 the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ile220Asp, with substantially similar results.

In Examples 51-52, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ile220Asp and Thr206Asp + Ile220Met, with substantially similar results.

Examples 53-57

Liquid Fabric Cleaning Compositions

	Example No.				
Component	53	54	55	56	57
Gly136Ser	0.05	0.03	0.30	0.03	0.10
Gly215Pro	–	–	–	0.01	0.20
C_{12}- C_{14} alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C_{10} alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly136Ser, with substantially similar results.

In Examples 56-57, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly136Ser and Gly215Pro, with substantially similar results.

Examples 58-59

Liquid Fabric Cleaning Composition

	Example No.	
Component	58	59
C_{12-14} alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C_{12-15} alkyl sulphate	8.0	8.0
Sodium sulfate of C_{12-15} alcohol 2 times ethoxylated	-	3.0
C_{12-15} alcohol 7 times ethoxylated	-	8.0
C_{12-15} alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Pro204Asn + Thr211Pro + Gly214Gln + Gly215Glu + Thr217Ser	0.2	0.2
Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.4	0.1
Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Pro204Asn + Thr211Pro + Gly214Gln + Gly215Glu + Thr217Ser, with substantially similar results.

Examples 60-62

Liquid Fabric Cleaning Composition

	Example No.		
Component	60	61	62
Citric Acid	7.10	3.00	3.00
Fatty Acid	2.00	-	2.00
Ethanol	1.93	3.20	3.20
Boric Acid	2.22	3.50	3.50
Monoethanolamine	0.71	1.09	1.09
1,2 Propanediol	7.89	8.00	8.00
NaCumene Sulfonate	1.80	3.00	3.00
NaFormate	0.08	0.08	0.08
NaOH	6.70	3.80	3.80
Silicon anti-foam agent	1.16	1.18	1.18
Leu96Met + Asn99Ser + Ser105Asp	0.0145	-	-
Thr211Ser + Gly222Asp	-	0.0145	-
Tyr169Ser + Ser190Asp + Ile220Thr	-	-	0.0145
Lipase	0.200	0.200	0.200
Cellulase	-	7.50	7.50
Soil release polymer	0.29	0.15	0.15
Anti-foaming agents	0.06	0.085	0.085
Brightener 36	0.095	-	-
Brightener 3	-	0.05	0.05
C_{12} alkyl benzenesulfonic acid	9.86	-	-
C_{12-15} alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
C_{12} glucose amide	-	5.00	5.00
C_{12-13} alkyl polyethoxylate (9)	2.00	2.00	2.00
Water, perfume and minors	balance to 100%		

c. Bar fabric cleaning compositions

Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 63-66

Bar Fabric Cleaning Compositions

Component	Example No. 63	64	65	66
Leu96Ala + Asp97Glu + Ser101Asp + Ile107Asp	0.3	-	0.1	0.02
Ser193Asp	-	-	0.4	0.03
C_{12}-C_{16} alkyl sulfate, Na	20.0	20.0	20.0	20.00
C_{12}-C_{14} N-methyl glucamide	5.0	5.0	5.0	5.00
C_{11}-C_{13} alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
$CaSO_4$	1.0	1.0	1.0	1.00
$MgSO_4$	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as $CaCO_3$, talc, clay, silicates, and the like.

In Examples 63-64 the Proteinase K variants recited in Tables 2-36, among others, are substituted for Leu 96Ala + Asp 97Glu + Ser101Asp + Ile107Asp, with substantially similar results.

In Examples 65-66, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Leu96Ala + Asp97Glu + Ser101Asp + Ile107Asp and Ser193Asp, with substantially similar results.

Examples 67-70
Bar Fabric Cleaning Compositions

	Example No.			
Component	67	68	69	70
Arg64Asp + Gly68Gln	0.3	-	0.1	0.02
Tyr137Gln	-	0.3	0.4	0.03
C_{12}-C_{16} alkyl sulfate, Na	20.0	20.0	20.0	20.00
C_{12}-C_{14} N-methyl glucamide	5.0	5.0	5.0	5.00
C_{11}-C_{13} alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
$CaSO_4$	1.0	1.0	1.0	1.00
$MgSO_4$	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as $CaCO_3$, talc, clay. silicates, and the like.

In Example 67, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Arg64Asp + Gly68Gln, with substantially similar results.

In Example 68, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Tyr137Gln, with substantially similar results.

In Examples 69-70, any combination of the Proteinase K variants recited in Tables 2-36, among others, are substituted for Arg64Asp + Gly68Glnand Tyr137Gln, with substantially similar results.

B. Additional Cleaning Compositions

In addition to the hard surface cleaning; dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning compositions where hydrolysis of an insoluble substrate is desired. Such

additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

1. Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning components of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991; all of which are incorporated herein by reference.

The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 71-74

Dentifrice Composition

Component	Example No. 71	72	73	74
Gly160Pro	2.000	3.500	1.500	2.000
Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
PEG-6*	1.000	1.000	1.000	1.000
Silica dental abrasive**	20.000	20.000	20.000	20.000
Sodium fluoride	0.243	0.243	0.243	0.243
Titanium dioxide	0.500	0.500	0.500	0.500
Sodium saccharin	0.286	0.286	0.286	0.286
Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
Flavor	1.040	1.040	1.040	1.040
Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
Carrageenan****	0.800	0.800	0.800	0.800
Water	balance to 100%			

*PEG-6 = Polyethylene glycol having a molecular weight of 600.

**Precipitated silica identified as Zeodent 119 offered by J.M. Huber.

***Carbopol offered by B.F. Goodrich Chemical Company.

****Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly160Pro, with substantially similar results.

Examples 75-78

Mouthwash Composition

	Example No.			
Component	75	76	77	78
Ala166Gln + Ser216Asp	3.00	7.50	1.00	5.00
SDA 40 Alcohol	8.00	8.00	8.00	8.00
Flavor	0.08	0.08	0.08	0.08
Emulsifier	0.08	0.08	0.08	0.08
Sodium Fluoride	0.05	0.05	0.05	0.05
Glycerin	10.00	10.00	10.00	10.00
Sweetener	0.02	0.02	0.02	0.02
Benzoic acid	0.05	0.05	0.05	0.05
Sodium hydroxide	0.20	0.20	0.20	0.20
Dye	0.04	0.04	0.04	0.04
Water	balance to 100%			

In Examples 75-78, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Ala166Gln + Ser216Asp, with substantially similar results.

Examples 79-82

Lozenge Composition

	Example No.			
Component	79	80	81	82
Gly70Ser + Ile107Gly + Leu133Met + Phe192His + Asn194Asp	0.01	0.03	0.10	0.02
Sorbitol	17.50	17.50	17.50	17.50
Mannitol	17.50	17.50	17.50	17.50
Starch	13.60	13.60	13.60	13.60
Sweetener	1.20	1.20	1.20	1.20
Flavor	11.70	11.70	11.70	11.70
Color	0.10	0.10	0.10	0.10
Corn Syrup	balance to 100%			

In Examples 79-82, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly70Ser + Ile107Gly + Leu133Met + Phe192His + Asn194Asp, with substantially similar results.

Examples 83-86
Chewing Gum Composition

Component	Example No. 83	84	85	86
Gly214Asn	0.03	0.02	0.10	0.05
Sorbitol crystals	38.44	38.40	38.40	38.40
Paloja-T gum base*	20.00	20.00	20.00	20.00
Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
Mannitol	10.00	10.00	10.00	10.00
Glycerine	7.56	7.56	7.56	7.56
Flavor	1.00	1.00	1.00	1.00

*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly214Asn, with substantially similar results.

2. Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention. Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier. Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young, incorporated herein by reference), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 87-90

Two-layer Effervescent Denture Cleansing Tablet

	Example No.			
Component	87	88	89	90
Acidic Layer				
Tyr137Leu + Tyr169Cys + Phe192Asn + Ser207Asp + Arg218Glu + Ile220Leu	1.0	1.5	0.01	0.05
Tartaric acid	24.0	24.0	24.00	24.00
Sodium carbonate	4.0	4.0	4.00	4.00
Sulphamic acid	10.0	10.0	10.00	10.00
PEG 20,000	4.0	4.0	4.00	4.00
Sodium bicarbonate	24.5	24.5	24.50	24.50
Potassium persulfate	15.0	15.0	15.00	15.00
Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
Pyrogenic silica	2.0	2.0	2.00	2.00
TAED*	7.0	7.0	7.00	7.00
Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
Flavor	1.0	1.0	1.00	1.00
Alkaline Layer				
Sodium perborate monohydrate	32.0	32.0	32.00	32.00
Sodium bicarbonate	19.0	19.0	19.00	19.00
EDTA	3.0	3.0	3.00	3.00
Sodium tripolyphosphate	12.0	12.0	12.00	12.00
PEG 20,000	2.0	2.0	2.00	2.00
Potassium persulfate	26.0	26.0	26.00	26.00
Sodium carbonate	2.0	2.0	2.00	2.00
Pyrogenic silica	2.0	2.0	2.00	2.00
Dye/flavor	2.0	2.0	2.00	2.00

*Tetraacetylethylene diamine

In Examples 87-90, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Tyr137Leu + Tyr169Cys + Phe192Asn + Ser207Asp + Arg218Glu + Ile220Leu, with substantially similar results.

3. Contact Lens Cleaning Compositions

In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective

amount of one or more of the enzyme variants, preferably from about 0.01% to about 50% of one or more of the enzyme variants, more preferably from about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier. Various contact lens cleaning composition formats such as tablets, liquids and the like are well known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith, issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August 25, 1981; each of which are incorporated herein by reference), and are generally appropriate for incorporation of one or more enzyme variants of the present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 91-94

Enzymatic Contact Lens Cleaning Solution

	Example No.			
Component	91	92	93	94
Gly102Gln	0.01	0.5	0.1	2.0
Glucose	50.00	50.0	50.0	50.0
Nonionic surfactant (polyoxyethlene-polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
Anionic surfactant (polyoxyethylene-alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
Sodium chloride	1.00	1.0	1.0	1.0
Borax	0.30	0.3	0.3	0.3
Water	balance to 100%			

In Examples 91-94, the Proteinase K variants recited in Tables 2-36, among others, are substituted for Gly102Gln, with substantially similar results.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the

appended claims, all such modifications that are within the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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RUBINGH, DONN N

(ii) TITLE OF INVENTION: PROTEINASE K VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

(iii) NUMBER OF SEQUENCES: 1

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(v) COMPUTER READABLE FORM:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Ala Gln Thr Asn Ala Pro Trp Gly Leu Ala Arg Ile Ser Ser Thr
1 5 10 15

Ser Pro Gly Thr Ser Thr Tyr Tyr Tyr Asp Glu Ser Ala Gly Gln Gly
20 25 30

```
Ser Cys Val Tyr Val Ile Asp Thr Gly Ile Glu Ala Ser His Pro Glu
        35                  40                  45

Phe Glu Gly Arg Ala Gln Met Val Lys Thr Tyr Tyr Tyr Ser Ser Arg
    50                  55                  60

Asp Gly Asn Gly His Gly Thr His Cys Ala Gly Thr Val Gly Ser Arg
65                  70                  75                  80

Thr Tyr Gly Val Ala Lys Lys Thr Gln Leu Phe Gly Val Lys Val Leu
                85                  90                  95

Asp Asp Asn Gly Ser Gly Gln Tyr Ser Thr Ile Ile Ala Gly Met Asp
            100                 105                 110

Phe Val Ala Ser Asp Lys Asn Asn Arg Asn Cys Pro Lys Gly Val Val
        115                 120                 125

Ala Ser Leu Ser Leu Gly Gly Gly Tyr Ser Ser Ser Val Asn Ser Ala
    130                 135                 140

Ala Ala Arg Leu Gln Ser Ser Gly Val Met Val Ala Val Ala Ala Gly
145                 150                 155                 160

Asn Asn Asn Ala Asp Ala Arg Asn Tyr Ser Pro Ala Ser Glu Pro Ser
                165                 170                 175

Val Cys Thr Val Gly Ala Ser Asp Arg Tyr Asp Arg Arg Ser Ser Phe
            180                 185                 190

Ser Asn Tyr Gly Ser Val Leu Asp Ile Phe Gly Pro Gly Thr Ser Ile
        195                 200                 205

Leu Ser Thr Trp Ile Gly Gly Ser Thr Arg Ser Ile Ser Gly Thr Ser
    210                 215                 220

Met Ala Thr Pro His Val Ala Gly Leu Ala Ala Tyr Leu Met Thr Leu
225                 230                 235                 240

Gly Lys Thr Thr Ala Ala Ser Ala Cys Arg Tyr Ile Ala Asp Thr Ala
                245                 250                 255

Asn Lys Gly Asp Leu Ser Asn Ile Pro Phe Gly Thr Val Asn Leu Leu
            260                 265                 270

Ala Tyr Asn Asn Tyr Gln Ala
        275
```

What is Claimed is:

1. A Proteinase K variant having a modified amino acid sequence of Proteinase K wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

A. when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 64, 65, 66, 68, 70 or 71; wherein

a. when a substitution occurs at position 64, the substituting amino acid is Asp or Glu;

b. when a substitution occurs at position 65, the substituting amino acid is Glu;

c. when a substitution occurs at position 66, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

d. when a substitution occurs at position 68, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

e. when a substitution occurs at position 70, the substituting amino acid is Asn. Asp. Gln. Glu. Pro or Ser; and

f. when a substitution occurs at position 71, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

B. when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 95, 96, 97, 98, 100, 102, 103, 104, 106 or 107; wherein

a. when a substitution occurs at position 95, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

b. when a substitution occurs at position 96, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser , Thr or Val;

c. when a substitution occurs at position 97, the substituting amino acid is Glu;

d. when a substitution occurs at position 98, the substituting amino acid is Glu;

e. when a substitution occurs at position 100, the substituting amino acid is Asn, Gln, Pro or Ser;

f. when a substitution occurs at position 102, the substituting amino acid is Asn, Gln, Glu, Pro or Ser;

g. when a substitution occurs at position 103, the substituting amino acid is Asn or Ser;

h. when a substitution occurs at position 104, the substituting amino acid is Asp, His, Ile, Leu, Met, Pro or Val;

i. when a substitution occurs at position 106, the substituting amino acid is Asn, Gln, Gly, Pro or Ser; and

j. when a substitution occurs at position 107, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Leu, Met, Pro, Ser, Thr or Val;

C. when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 133, 134, 136, 137, 138, 139 or 140; wherein

a. when a substitution occurs at position 133, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Met, Pro, Ser, Thr or Val;

b. when a substitution occurs at position 134, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

c. when a substitution occurs at position 136, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

d. when a substitution occurs at position 137, the substituting amino acid is Asp, His, Ile, Leu, Met or Pro;

e. when a substitution occurs at position 138, the substituting amino acid is Asp or Glu;

f. when a substitution occurs at position 139, the substituting amino acid is Asp or Glu; and

g. when a substitution occurs at position 140, the substituting amino acid is Asp or Glu;

D. when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 161, 162, 163, 164, 165, 166, 167, 168, 169 or 170; wherein

a. when a substitution occurs at position 161, the substituting amino acid is Gln;

b. when a substitution occurs at position 162, the substituting amino acid is Asp, Gln, Glu or Ser;

c. when a substitution occurs at position 163, the substituting amino acid is Asp, Gln, Glu or Ser;

d. when a substitution occurs at position 164, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

e. when a substitution occurs at position 165, the substituting amino acid is Glu;

f. when a substitution occurs at position 166, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

g. when a substitution occurs at position 167, the substituting amino acid is Asp or Glu;

h. when a substitution occurs at position 168, the substituting amino acid is Asp, Gln, Glu or Ser;

i. when a substitution occurs at position 169, the substituting amino acid is Asp, His, Ile, Leu, Met or Pro; and

j. when a substitution occurs at position 170, the substituting amino acid is Asp or Glu;

E. when a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 190, 192, 193 or 194; wherein

a. when a substitution occurs at position 190, the substituting amino acid is Asp or Glu;

b when a substitution occurs at position 192, the substituting amino acid is Asn, Cys, Gln, His, Ile, Met, Pro, Thr, Tyr or Val;

c. when a substitution occurs at position 193, the substituting amino acid is Asp or Glu; and

d. when a substitution occurs at position 194, the substituting amino acid is Asp, Gln, Glu or Ser; and

F. when a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 203, 204, 205, 206, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222 or 223; wherein

a. when a substitution occurs at position 203, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

b. when a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;

c. when a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

d. when a substitution occurs at position 206, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

e. when a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;

f. when a substitution occurs at position 209, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;

g. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

h. when a substitution occurs at position 211, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

i. when a substitution occurs at position 212, the substituting amino acid is Asp or Glu;

j. when a substitution occurs at position 213, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;

k. when a substitution occurs at position 214, the substituting amino acid is Asn, Gln, Pro or Ser;

l. when a substitution occurs at position 215, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

m. when a substitution occurs at position 216, the substituting amino acid is Asp or Glu;

n. when a substitution occurs at position 217, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

o. when a substitution occurs at position 218, the substituting amino acid is Asp or Glu;

p. when a substitution occurs at position 219, the substituting amino acid is Asp or Glu;

q. when a substitution occurs at position 220, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;

r. when a substitution occurs at position 221, the substituting amino acid is Asp or Glu;

s. when a substitution occurs at position 222, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and

t. when a substitution occurs at position 223, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

whereby the Proteinase K variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type Proteinase K.

2. The Proteinase K variant of Claim 1, wherein one or more substitutions occur in the first loop region.

3. The Proteinase K variant of Claim 1, wherein one or more substitutions occur in the second loop region.

4. The Proteinase K variant of Claim 1, wherein one or more substitutions occur in the third loop region.

5. The Proteinase K variant of Claim 1, wherein one or more substitutions occur in the fourth loop region.

6. The Proteinase K variant of Claim 1, wherein one or more substitutions occur in the fifth loop region.

7. The Proteinase K variant of Claim 1, wherein one or more substitutions occur in the sixth loop region.

8. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, characterized in that the cleaning composition comprises the Proteinase K variant of any of Claims 1-7 and a cleaning composition carrier; preferably the cleaning composition is a hard surface cleaning composition or a fabric cleaning composition; preferably the composition comprises at least about 5% surfactant and at least about 5% builder, by weight of the composition; preferably the composition further comprises cleaning composition materials selected from the group consisting of solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds supressors, fabric softeners, suds boosters, enzyme stabilizers, bleaching agents, dyes, perfumes, and mixtures thereof.

9. A DNA sequence encoding the Proteinase K variant of any of Claims 1-7.

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(54) Title: PROTEINASE K VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

(57) Abstract

The present invention relates to proteinase K variants having a modified amino acid sequence of wild-type proteinase K amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type proteinase K (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the proteinase K variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type proteinase K. The present invention also relates to DNA sequences encoding such proteinase K variants. The present invention also relates to compositions comprising such proteinase K variants for cleaning a variety of surfaces.

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 96/03005

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/57 C12N9/58 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 405 901 A (UNILEVER PLC ;UNILEVER NV (NL)) 2 January 1991	1,2,8,9
Y	see page 15, line 21 - page 19, line 42	1,2,8,9

X	JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 18D, 1994, page 151 XP000651912 P. BRODE ET AL: "Surface active variants of subtilisin BPN': interfacial hydrolysis"	1,2,9
Y	see abstract S307	8

Y	WO 89 09819 A (GENEX CORP) 19 October 1989 see claim 1; table 2	1,2,8,9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 1997	30. 05. 97
Name and mailing address of the ISA	**Authorized officer**
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	VAN DER SCHAAL C.A.

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 96/03005

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROTEIN ENGINEERING, vol. 4, no. 7, 1 January 1991, pages 719-737, XP002008733 SIEZEN R J ET AL: "HOMOLOGY MODELLING AND PROTEIN ENGINEERING STRATEGY OF SUBTILASES, THE FAMILY OF SUBTILISIN-LIKE SERINE PROTEINASES" see the whole document ---	1,2,8,9
A	CHEMICAL ABSTRACTS, vol. 116, no. 23, 8 June 1992 Columbus, Ohio, US; abstract no. 230623, BRODE, P. F., III ET AL: "Subtilisin BPN': activity on an immobilized substrate" XP002027110 cited in the application see abstract & LANGMUIR (1992), 8(5), 1325-9 CODEN: LANGD5;ISSN: 0743-7463, 1992, ---	
P,X	WO 95 30011 A (PROCTER & GAMBLE) 9 November 1995 see the whole document ---	1,2,8,9
P,X	WO 95 30010 A (PROCTER & GAMBLE) 9 November 1995 see the whole document -----	1,2,8,9

3

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/US 96/03005

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

6 inventions * see continuation-sheet PCT/ISA/210 *

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

2 and, partially, 1,8,9

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 96/03005

FURTHER INFORMATION CONTINUED FROM PCT/ISA/

1. claim 2 and, partially, 1,8,9:

   Proteinase K variants having modified amino acids in the first loop region, DNA encoding and cleaning compositions containing the enzyme

2. claim 3 and, partially, 1,8,9:

   Proteinase K variants having modified amino acids in the second loop region, DNA encoding and cleaning compositions containing the enzyme

3. claim 4 and, partially, 1,8,9:

   Proteinase K variants having modified amino acids in the third loop region, DNA encoding and cleaning compositions containing the enzyme.

4. claim 5 and, partially, 1,8,9:

   Proteinase K variants having modified amino acids in the fourth loop region, DNA encoding and cleaning compositions containing the enzyme

5. claim 6 and, partially, 1,8,9:

   Proteinase K variants having modified amino acids in the fifth loop region, DNA encoding and cleaning compositions containing the enzyme

6. claim 7 and, partially, 1,8,9,:

   Proteinase K variants having modified amino acids in the sixth loop region, DNA encoding and cleaning compositions containing the enzyme

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 96/03005

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 96/03005

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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